US 20240209099A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2024/0209099 A1**

QIAN et al. (43) **Pub. Date: Jun. 27, 2024**

(54) **NOVEL ANTI-HVEGFR2 ANTIBODIES**

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(21) Appl. No.: **18/555,341**

(22) PCT Filed: **Apr. 13, 2022**

(86) PCT No.: **PCT/CN2022/086525**

§ 371 (c)(1),
(2) Date: **Oct. 13, 2023**

(30) **Foreign Application Priority Data**

Apr. 14, 2021 (WO) PCT/CN2021/087278

Publication Classification

(51) **Int. Cl.**

C07K 16/28	(2006.01)
A61K 39/00	(2006.01)
A61K 47/68	(2006.01)
A61K 49/00	(2006.01)
A61K 51/10	(2006.01)
A61P 35/00	(2006.01)
G01N 33/68	(2006.01)

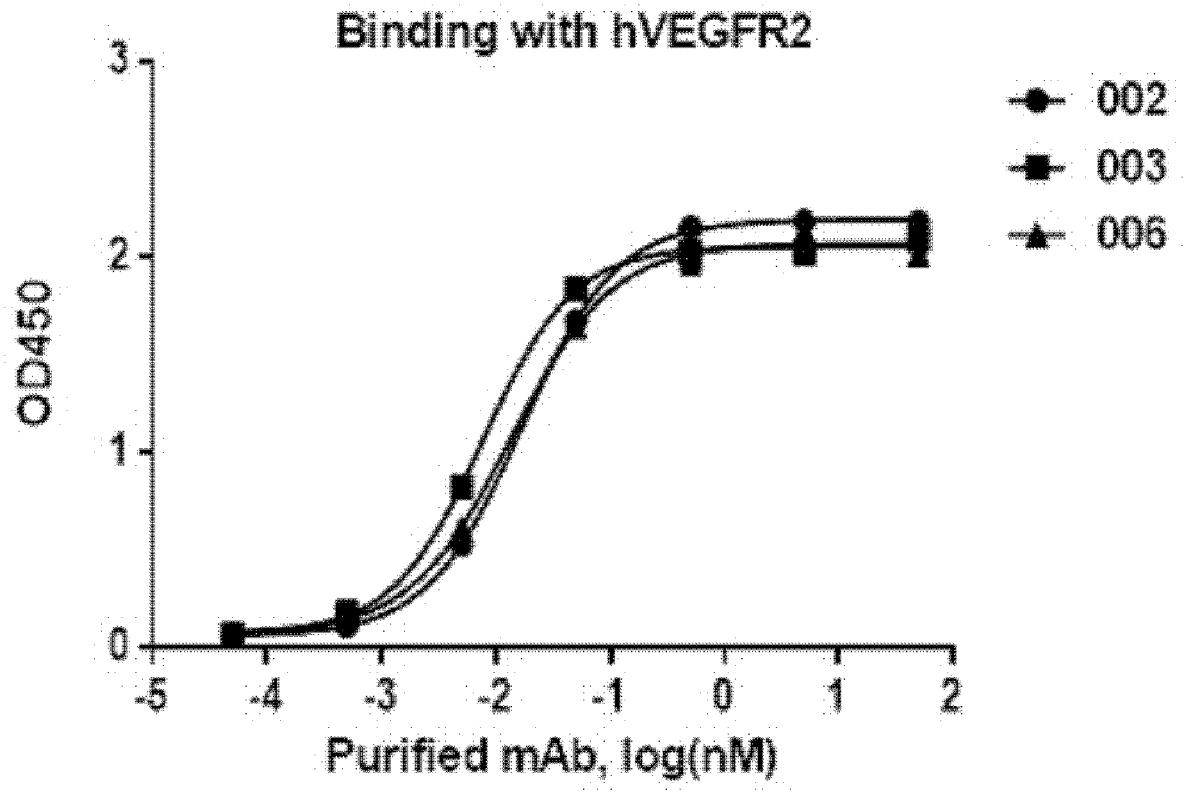
(52) **U.S. Cl.**

CPC ***C07K 16/2863*** (2013.01); ***A61K 47/6849*** (2017.08); ***A61K 49/0058*** (2013.01); ***A61K 51/103*** (2013.01); ***A61P 35/00*** (2018.01); ***G01N 33/6872*** (2013.01); *A61K 2039/505* (2013.01); *C07K 2317/24* (2013.01); *C07K 2317/31* (2013.01); *G01N 2333/475* (2013.01)

(57) **ABSTRACT**

Provided are anti-hVEGFR2 antibodies or antigen-binding fragments thereof, isolated polynucleotides encoding the same, pharmaceutical compositions comprising the same, and the uses thereof.

Specification includes a Sequence Listing.



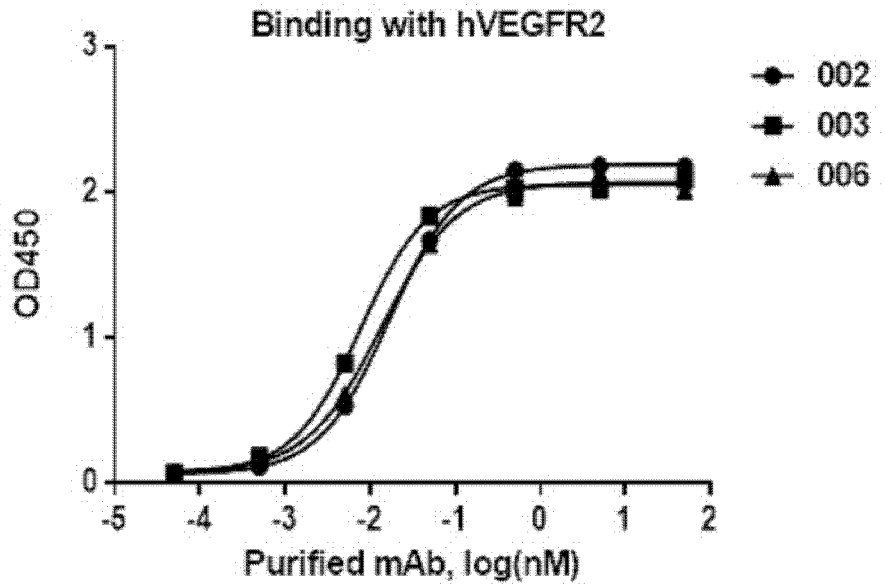

Binding with hVEGFR2
OD450
Purified mAb, log(nM)
002
003
006

FIG. 1A

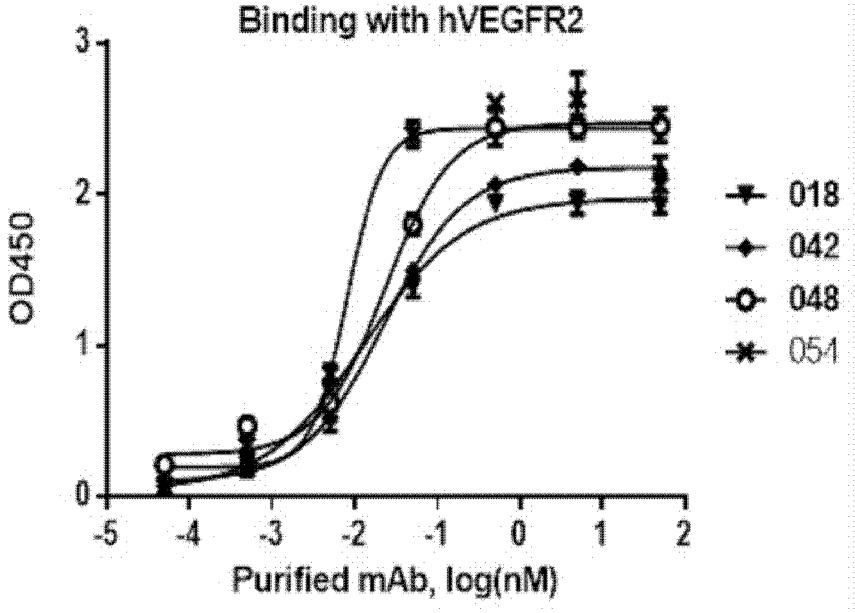
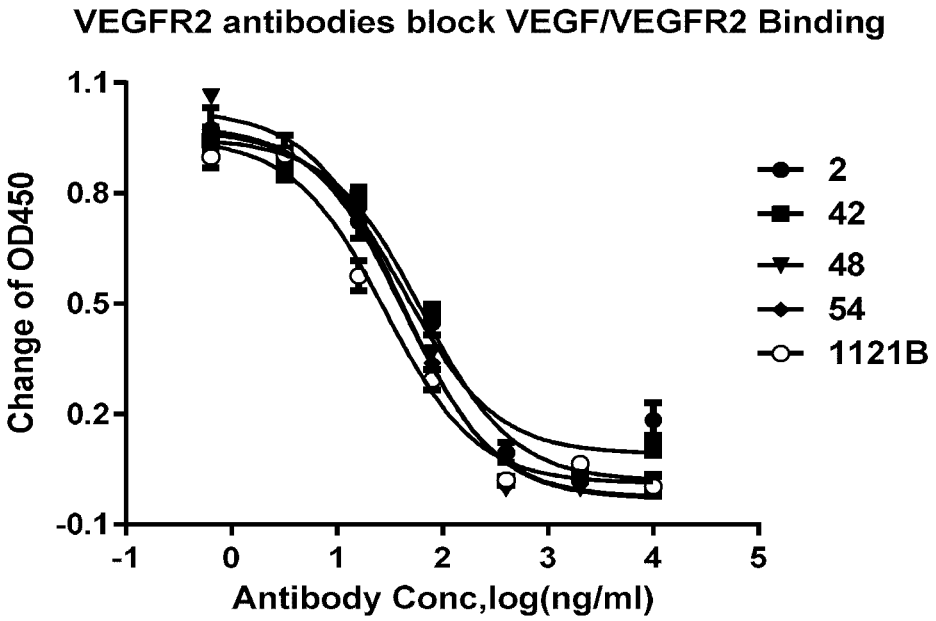
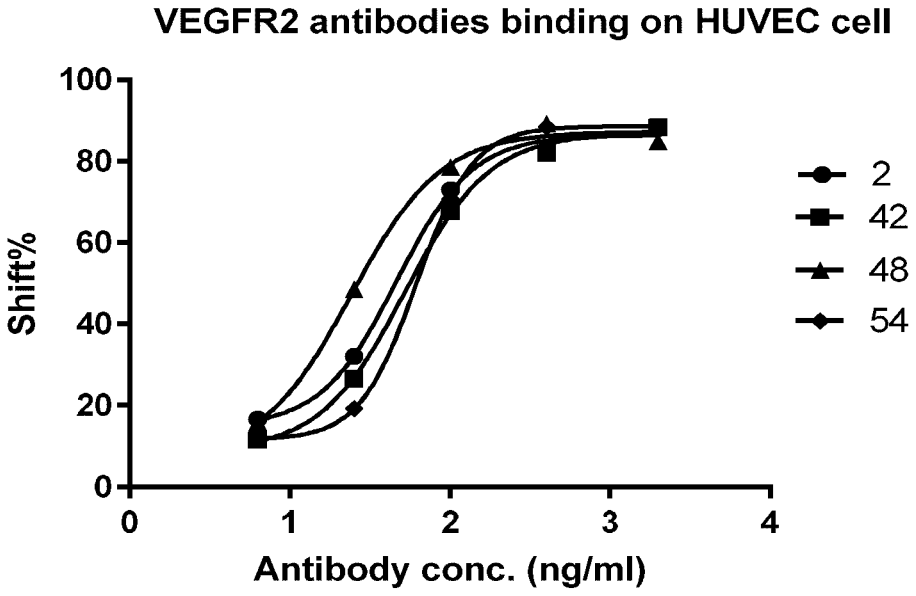

Binding with hVEGFR2
OD450
Purified mAb, log(nM)
018
042
048
051

FIG. 1B



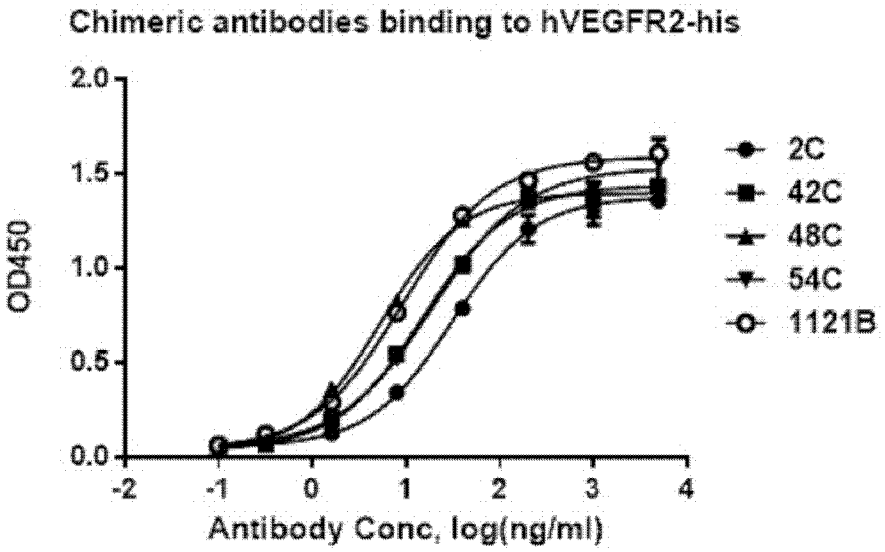
	2	42	48	54	1121B
IC50	43.41	62.93	41.65	43.32	28.53

FIG. 2



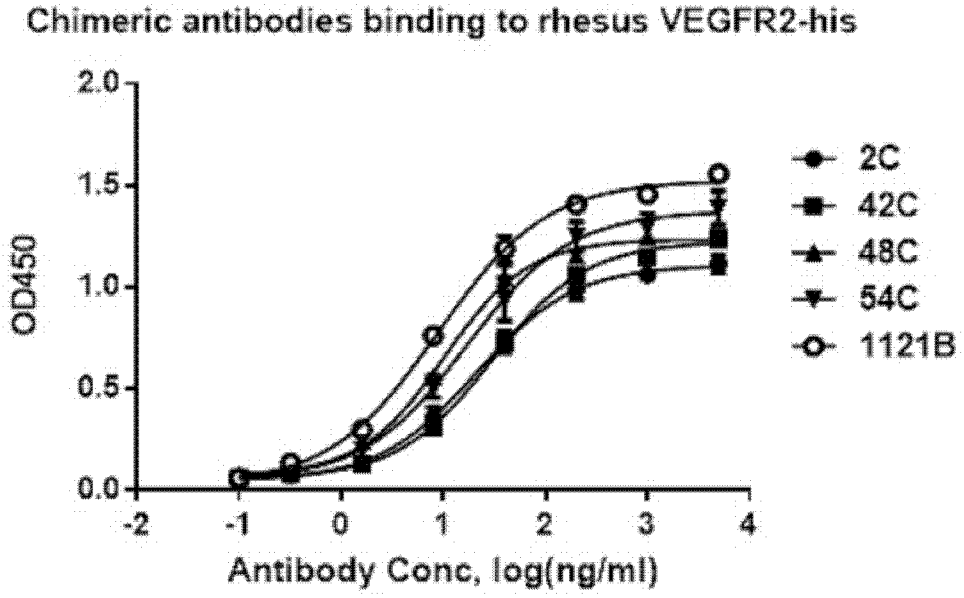
	2	42	48	54
EC50	46.18	52.03	23.96	61.24

FIG. 3



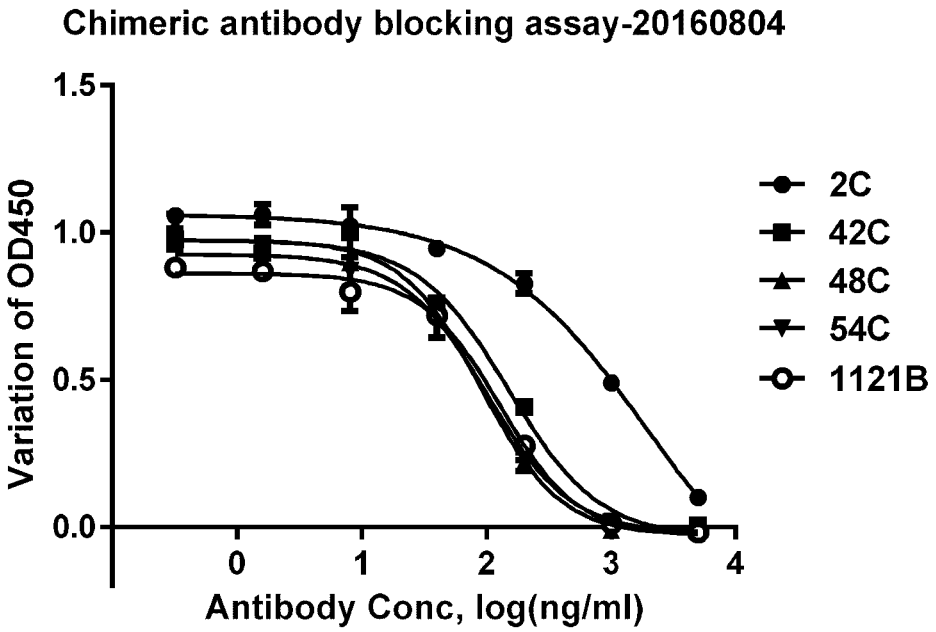
	2C	42C	48C	54C	1121B
EC50	30.84	14.99	5.303	18.87	9.263

FIG. 4A



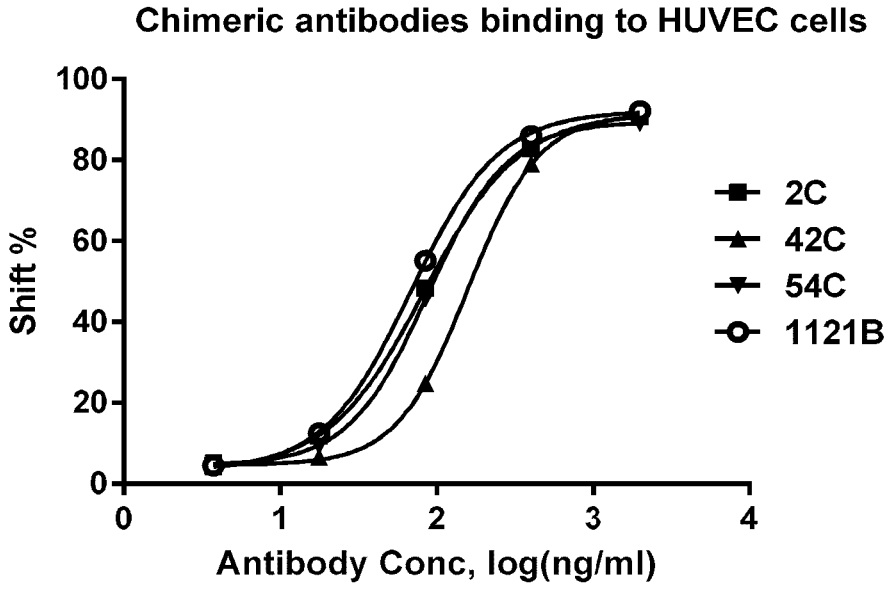
	2C	42C	48C	54C	1121B
EC50	20.90	29.94	10.13	16.81	8.798

FIG. 4B



	2C	42C	48C	54C	1121B
IC50	2106	148.0	89.98	100.2	126.4

FIG. 5



	2C	42C	54C	1121B
EC50	82.28	157.3	87.46	68.24

FIG. 6

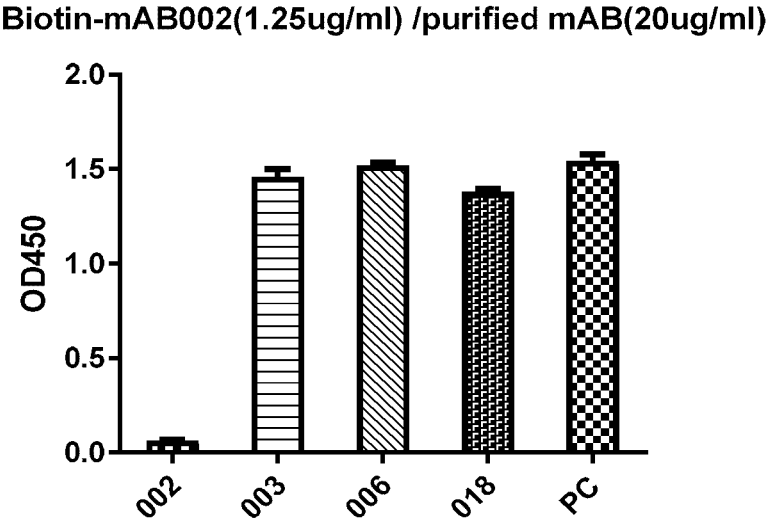

Biotin-mAB002(1.25ug/ml) /purified mAB(20ug/ml)
OD450
2.0
1.5
1.0
0.5
0.0
002
003
006
018
PC

FIG. 7A

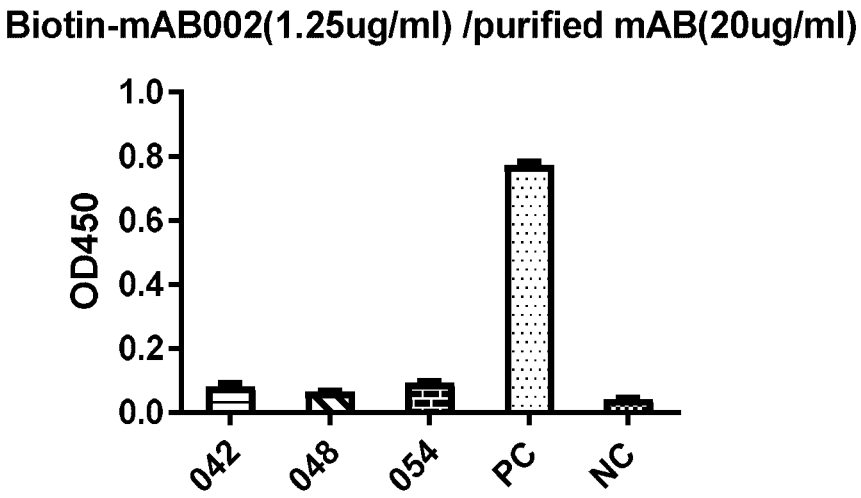

Biotin-mAB002(1.25ug/ml) /purified mAB(20ug/ml)
OD450
1.0
0.8
0.6
0.4
0.2
0.0
042
048
054
PC
NC

FIG. 7B

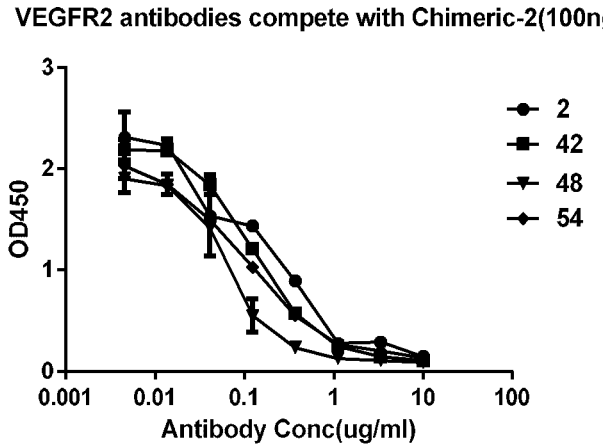


FIG. 8A

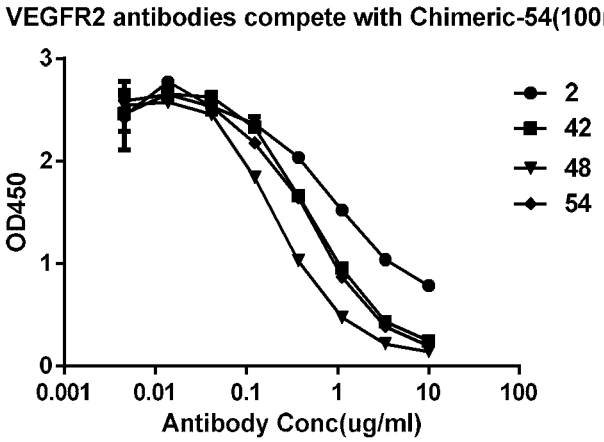


FIG. 8B

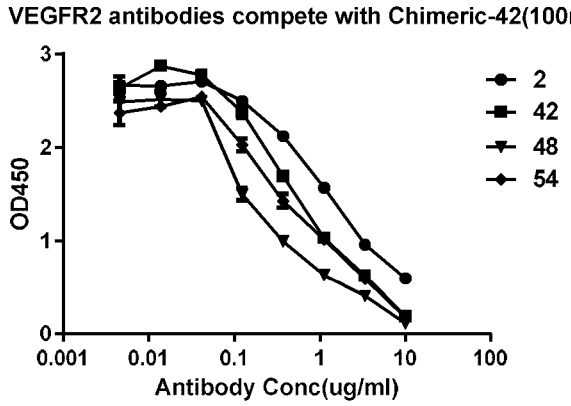


FIG. 8C

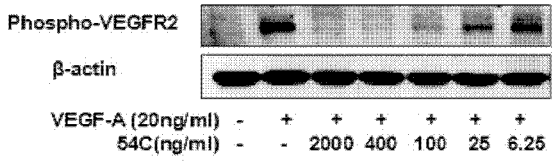


FIG. 9A

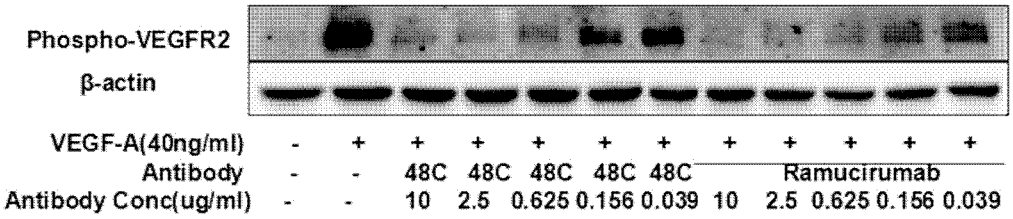


FIG. 9B

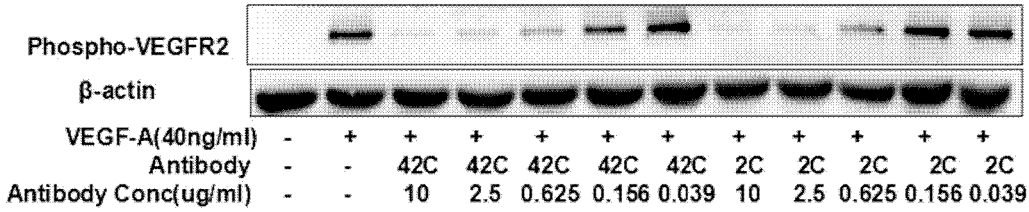
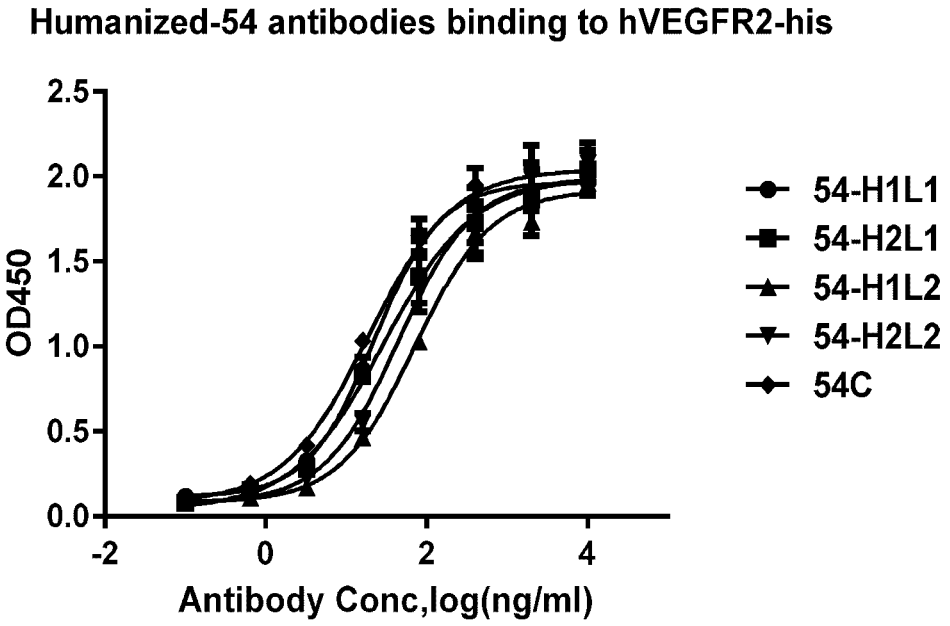
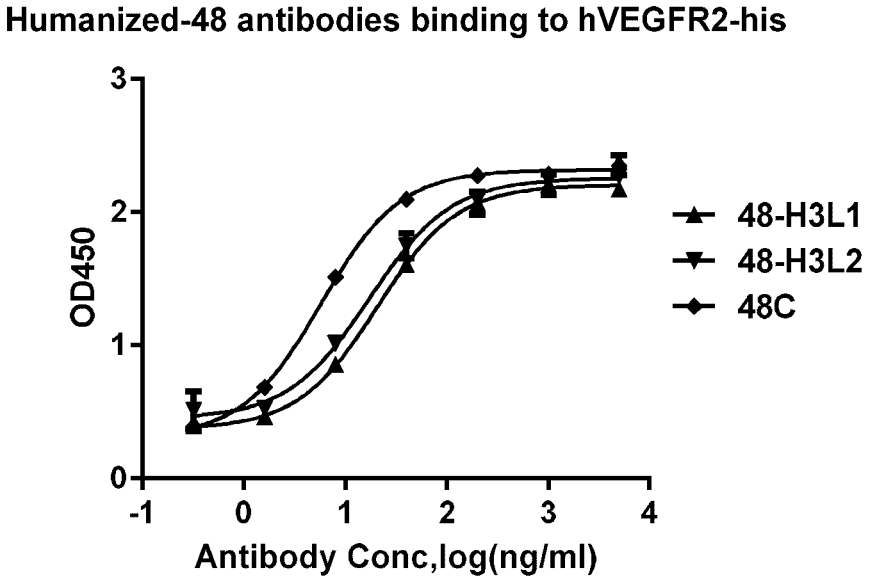


FIG. 9C



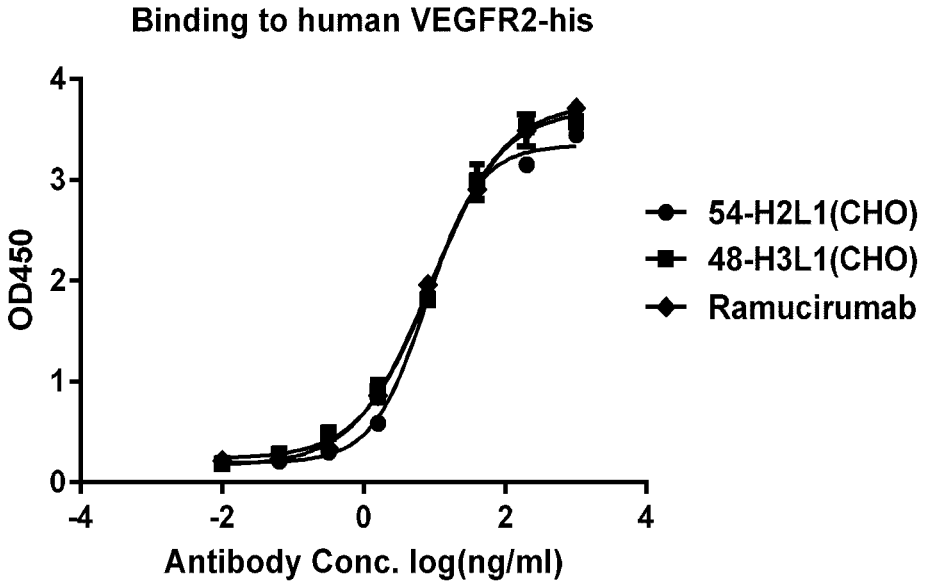
	54-H1L1	54-H2L1	54-H1L2	54-H2L2	54C
EC50	22.08	28.76	70.63	44.20	18.64

FIG. 10A



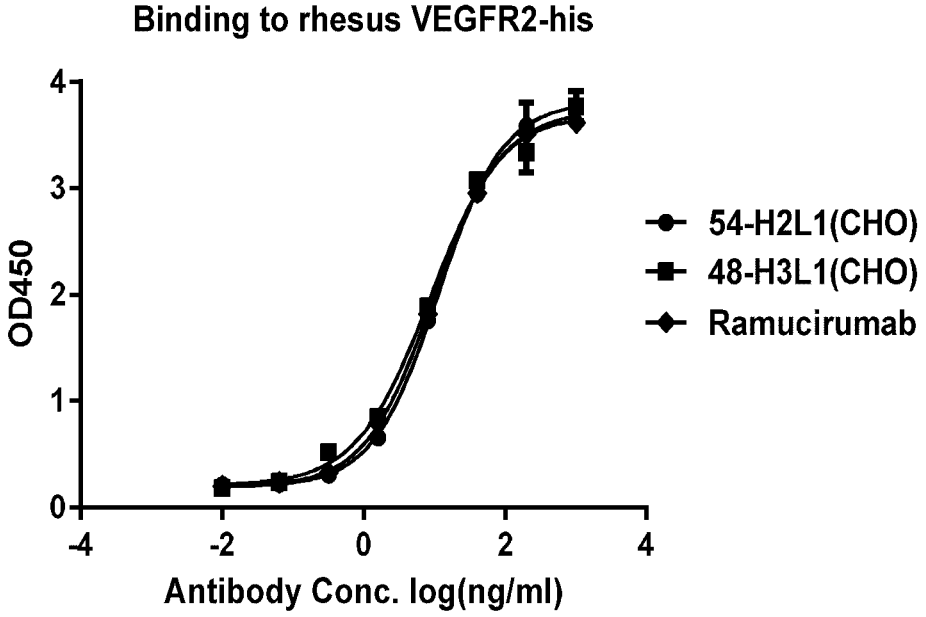
	48-H3L1	48-H3L2	48C
EC50	20.51	17.14	5.738

FIG. 10B



	54-H2L1(CHO)	48-H3L1(CHO)	Ramucirumab
EC50	7.548	8.99	8.707

FIG. 11A



	54-H2L1(CHO)	48-H3L1(CHO)	Ramucirumab
EC50	11.1	8.637	9.188

FIG. 11B

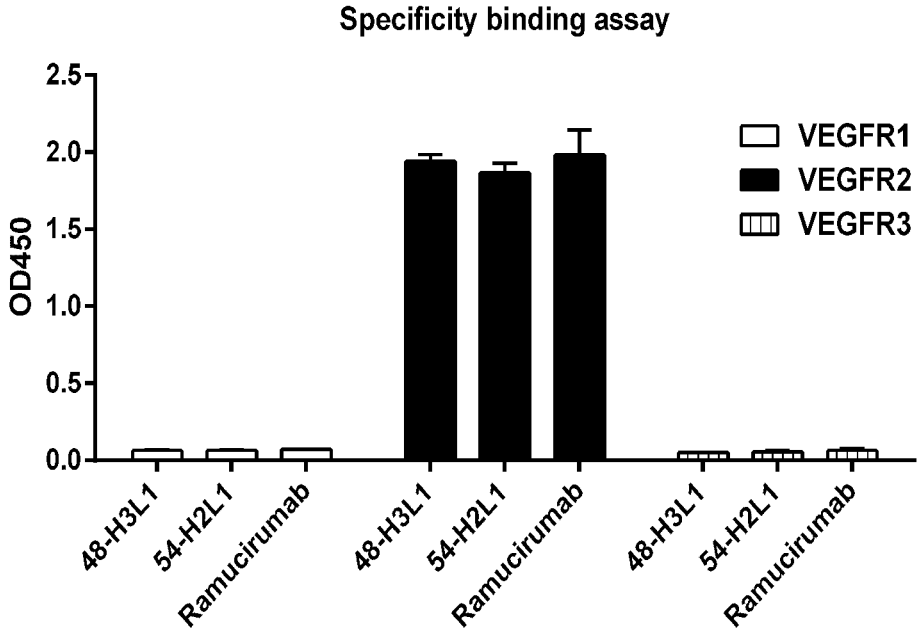
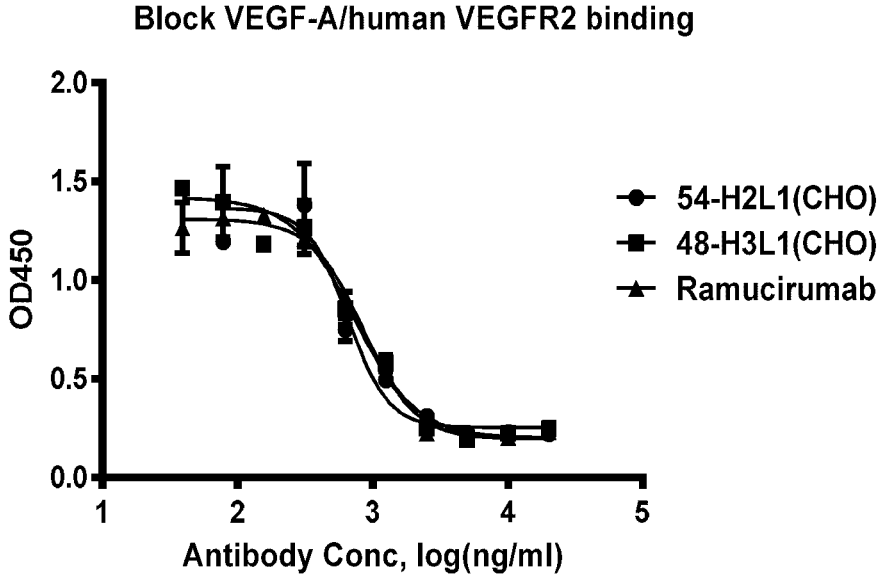
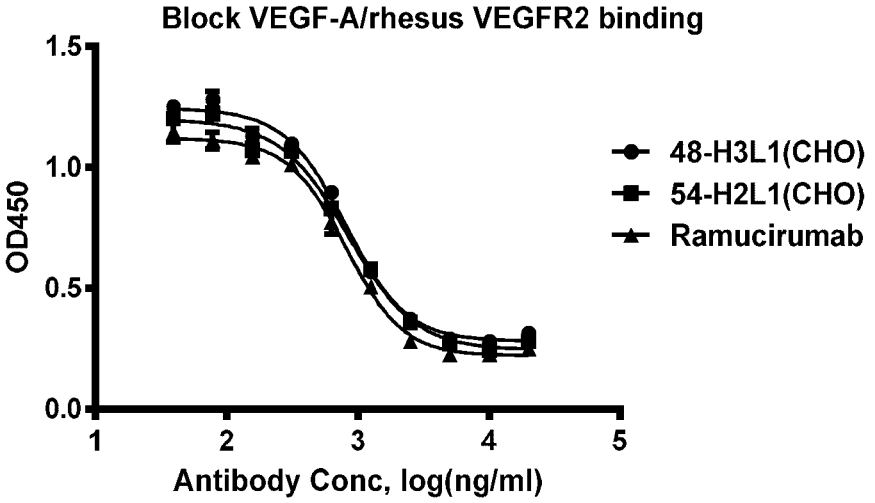


FIG. 12



	54-H2L1(CHO)	48-H3L1(CHO)	Ramucirumab
IC50	654.5	726.7	849.3

FIG. 13A



	54-H2L1(CHO)	48-H3L1(CHO)	Ramucirumab
IC50	805.1	832.5	804.7

FIG. 13B

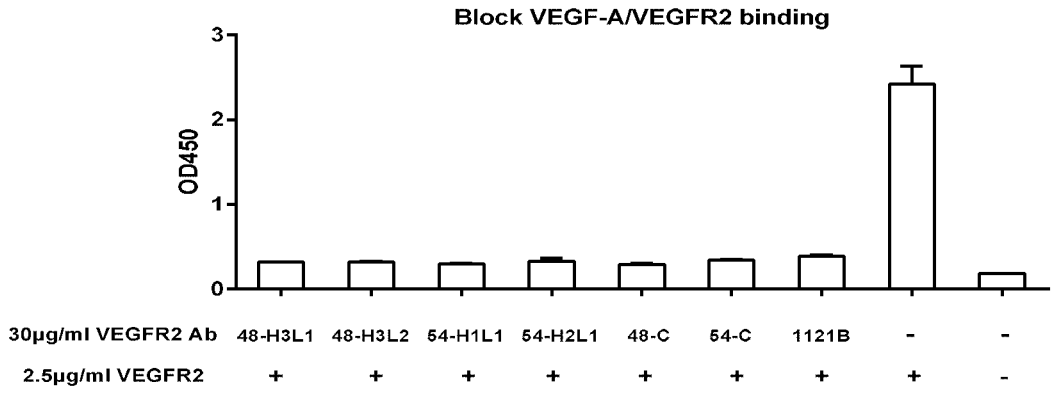


FIG. 14A

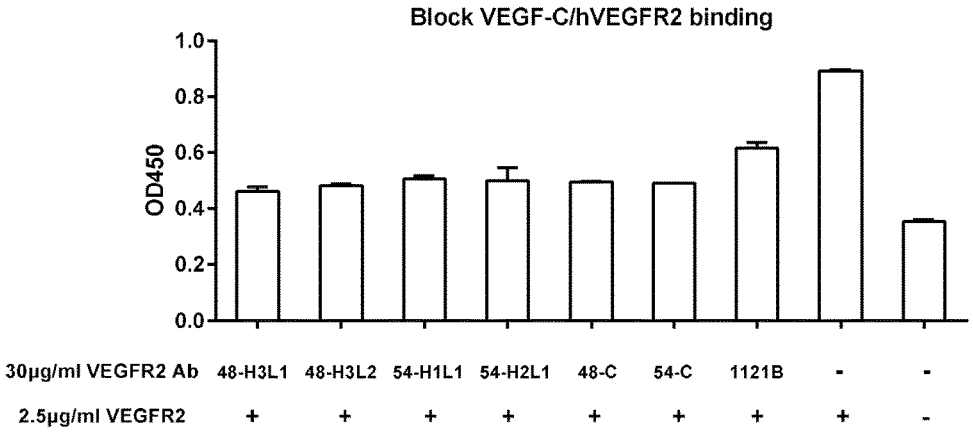


FIG. 14B

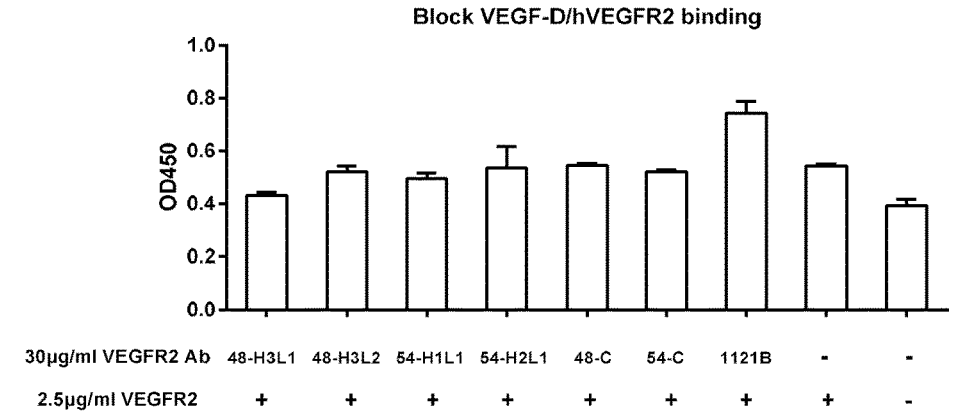


FIG. 14C

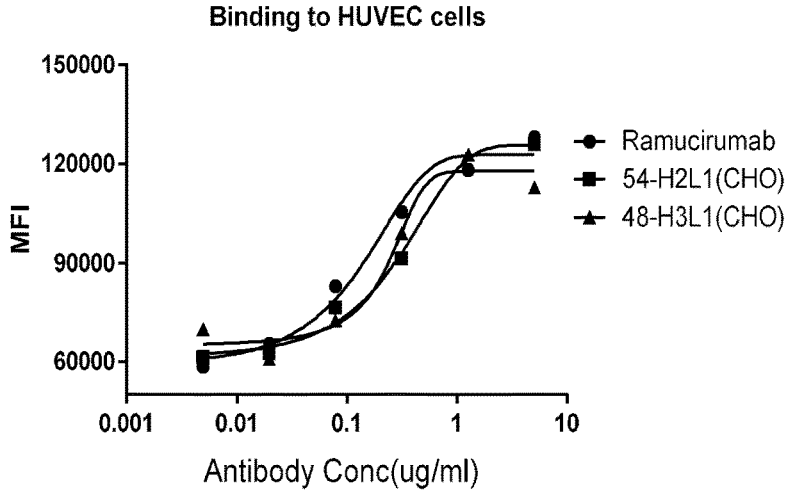


FIG. 15

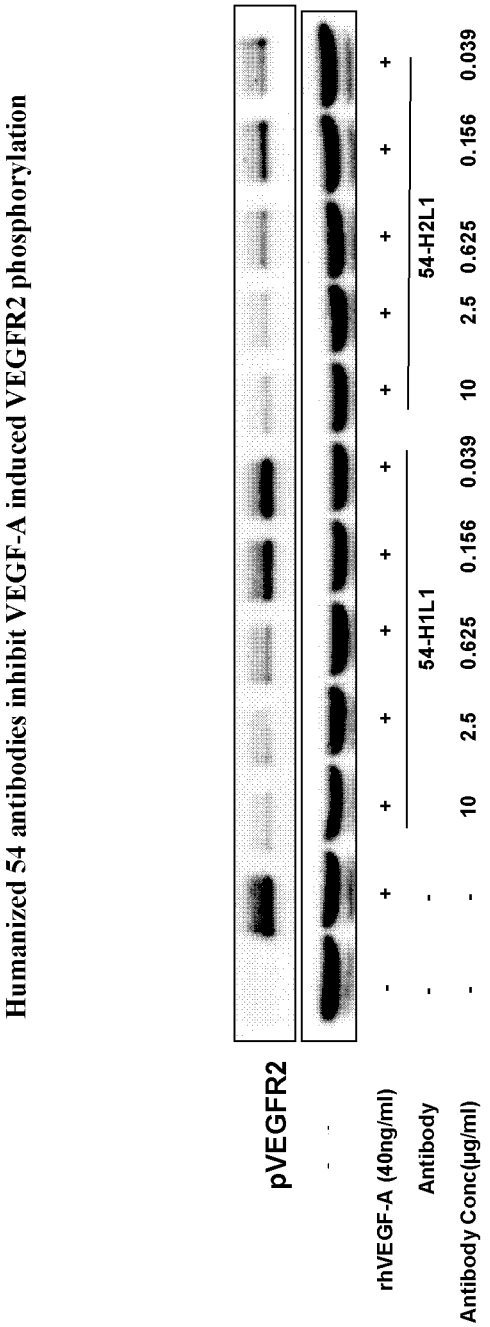
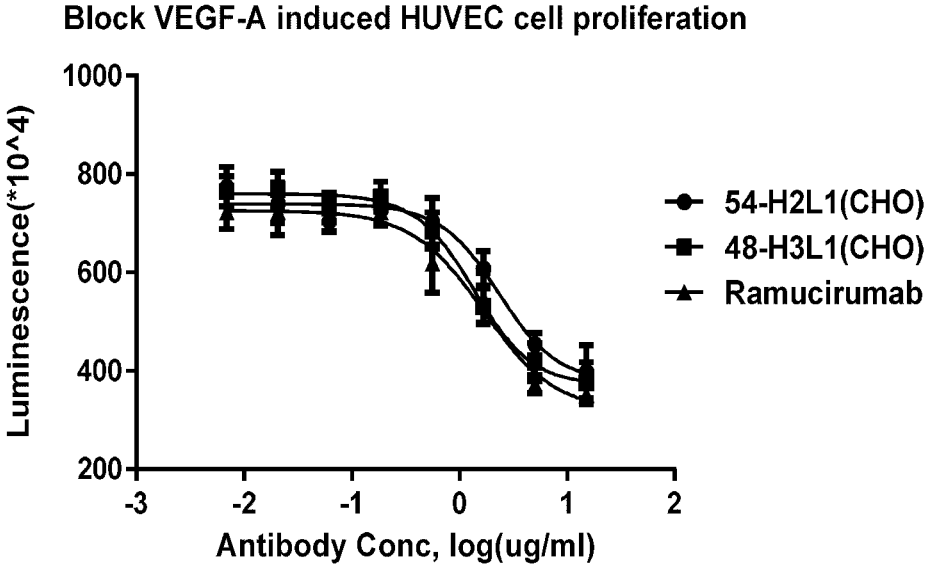
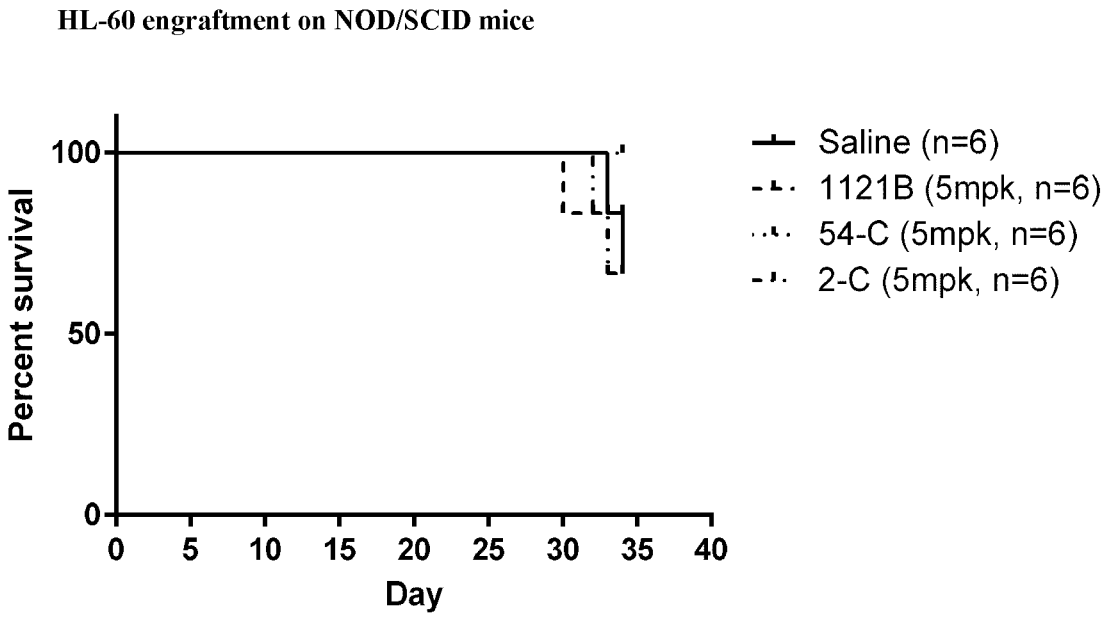

Humanized 54 antibodies inhibit VEGF-A induced VEGFR2 phosphorylation
pVEGFR2
rhVEGF-A (40ng/ml) - + + + + + + + + + + + +
Antibody - - 54-H1L1 54-H2L1
Antibody Conc(μg/ml) - - 10 2.5 0.625 0.156 0.039 10 2.5 0.625 0.156 0.039

FIG. 16



	54-H2L1(CHO)	48-H3L1(CHO)	Ramucirumab
IC50	2.291	1.356	1.660

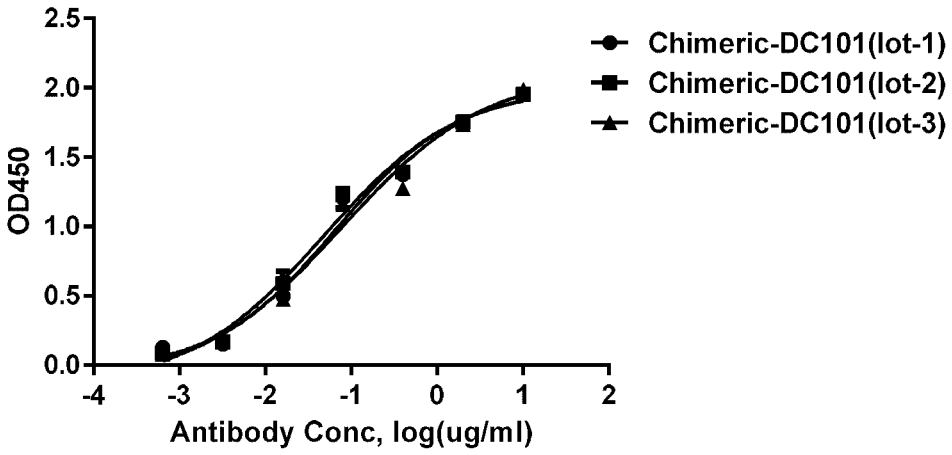
FIG. 17



1.5×10^7 HL-60 cell/mouse were injected intravenously.
3 days after inoculation mice wre intravenouslytreated saline, 1121B, 54-C and 2-C twice/week for 4 weeks.

FIG. 18

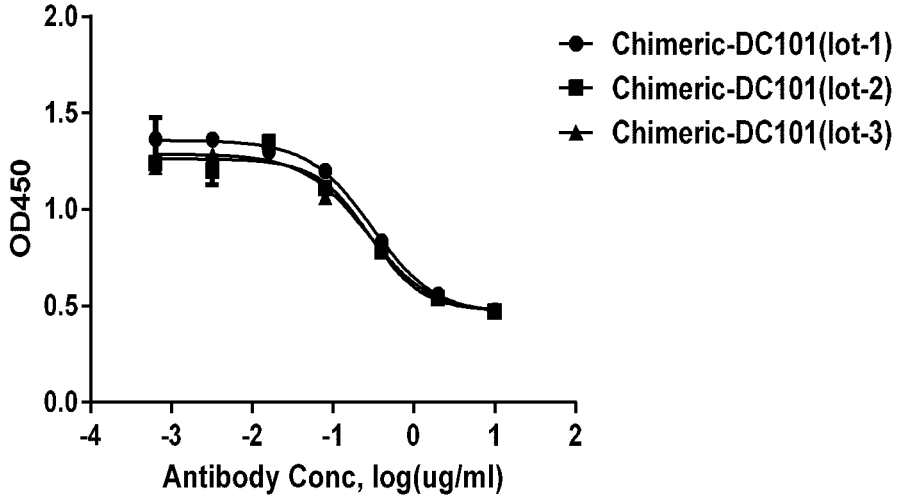
ELISA blocking assay was conducted to confirm its VEGF blockade activity.



	Chimeric-DC101(lot-1)	Chimeric-DC101(lot-2)	Chimeric-DC101(lot-3)
EC50	0.0645	0.04767	0.07641

FIG. 19

Blockade of mouse VEGFR2 binding to human VEGF165



	Chimeric-DC101(lot-1)	Chimeric-DC101(lot-2)	Chimeric-DC101(lot-3)
IC50	0.3006	0.2804	0.2652

FIG. 20

MKN45 xenograft tumor on nude mice (mean±S.E.M., n=10)

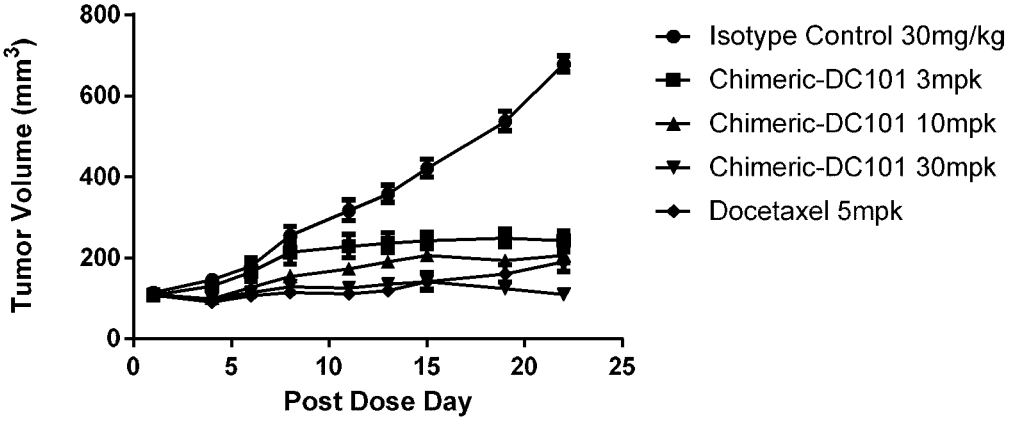


FIG. 21

H460 xenograft tumor on nude mice (mean±S.E.M., n=10)

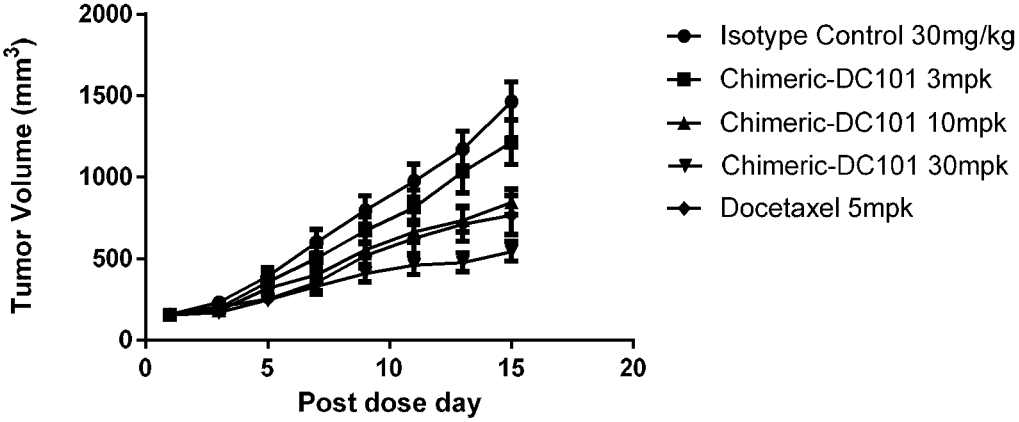


FIG. 22

H1975 xenograft tumor on nude mice (mean±S.E.M., n=10)

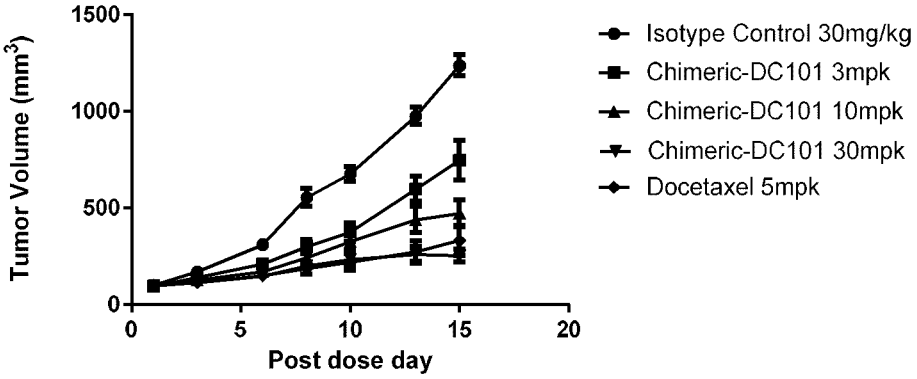


FIG. 23

NOVEL ANTI-HVEGFR2 ANTIBODIES

FIELD OF THE INVENTION

[0001] The present disclosure generally relates to novel anti-hVEGFR2 antibodies that specifically bind to human VEGFR2 (hVEGFR2).

BACKGROUND

[0002] Vascular endothelial growth factor receptor 2 (VEGFR2) is a type III tyrosine kinase that can be stimulated by vascular endothelial growth factor (VEGF) (e.g., VEGF-A, VEGF-C and VEGF-D). Upon binding to VEGF-A, VEGFR2 increases its expression and becomes dimerized form. The dimerization of VEGFR2 induces tyrosine phosphorylation of VEGFR2 followed by activation of downstream pathways involved in proliferation, migration, differentiation, tube formation, maintenance of vascular integrity and increase in vascular permeability of the endothelial cells. Abnormally high expression and/or activity of VEGFR2 disrupts the homeostasis of angiogenesis and leads to various cancers and/or angiogenic diseases.

[0003] Therefore, there exists significant needs for novel anti-hVEGFR2 antibodies which can be used for treatment of diseases positive for hVEGFR2 expression, such as cancers as well as other angiogenic diseases.

BRIEF SUMMARY OF THE INVENTION

[0004] Throughout the present disclosure, the articles "a," "an," and "the" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an antibody" means one antibody or more than one antibody.

[0005] The present disclosure provides, among others, novel monoclonal anti-hVEGFR2 antibodies, nucleotide sequences encoding such, and the uses thereof.

[0006] In one aspect, the present disclosure provides an anti-hVEGFR antibody or an antigen-binding fragment thereof, comprising heavy chain HCDR1, HCDR2 and HCDR3 and/or light chain LCDR1, LCDR2 and LCDR3 sequences, wherein:

[0007] the HCDR1 sequence comprises SSWMN (SEQ ID NO: 1), DYYMS (SEQ ID NO: 19), X_1YGMS (SEQ ID NO: 41), X_4YWIM (SEQ ID NO: 44), or a homologue sequence of at least 80% sequence identity thereof,

[0008] the HCDR2 sequence comprises RIFPGDGDTYYNGKFQV (SEQ ID NO: 2), FIRNKANGYTTEYSASVKG (SEQ ID NO: 20), SISX$_2$GGSYTYYADSVX$_{19}$G (SEQ ID NO: 42), DIYPGX$_5$GSTNYNEKFKS (SEQ ID NO: 45) or a homologue sequence of at least 80% sequence identity thereof,

[0009] the HCDR3 sequence comprises FLDTSGRYVDY (SEQ ID NO: 3), FDYYGSTYCFDY (SEQ ID NO: 21), EX$_3$DGNYDY (SEQ ID NO: 43), DSNPDY (SEQ ID NO: 46), or a homologue sequence of at least 80% sequence identity thereof;

[0010] the LCDR1 sequence comprises KASQDVNTAVA (SEQ ID NO: 4), RASQSVSTSSSSFMH (SEQ ID NO: 22), RSSKSLLYKDGKTYLN (SEQ ID NO: 28), RASESVX$_6$NSGISFMX$_7$ (SEQ ID NO: 47) or a homologue sequence of at least 80% sequence identity thereof,

[0011] the LCDR2 sequence comprises SASYRYI (SEQ ID NO: 5), YASNLES (SEQ ID NO: 23), LMSTRAS (SEQ ID NO: 29), AASX$_8$QX$_9$S (SEQ ID NO: 48) or a homologue sequence of at least 80% sequence identity thereof;

[0012] the LCDR3 sequence comprises QQHYRAPLT (SEQ ID NO: 6), QHTWEIPLT (SEQ ID NO: 24), QQLVEYPFT (SEQ ID NO: 30), QQSKEVPYT (SEQ ID NO: 49) or a homologue sequence of at least 80% sequence identity thereof;

[0013] wherein X1 is I or M, X_2 is V or I, X_3 is L or M, X_4 is T or S, X_5 is T or S, X_6 is D or E, X_7 is T or H, X_8 is T or Y, X_9 is G or R, and X_{19} is E or K.

[0014] In certain embodiments, the HCDR1 comprises an amino acid sequence of SEQ ID NO: 41, the HCDR2 comprises an amino acid sequence of SEQ ID NO: 42, the HCDR3 comprises an amino acid sequence of SEQ ID NO: 43, the LCDR1 comprises a sequence of SEQ ID NO: 28, the LCDR2 comprises a sequence of SEQ ID NO: 29, and the LCDR3 comprises a sequence of SEQ ID NO: 30.

[0015] In certain embodiments, in the antibody or an antigen-binding fragment thereof provided herein, wherein

[0016] a) the HCDR1 comprises the sequence of SEQ ID NO: 25, a HCDR2 comprises the sequence of SEQ ID NO: 26, the HCDR3 comprises the sequence of SEQ ID NO: 27; the LCDR1 comprises the sequence of SEQ ID NO: 28, the LCDR2 comprises the sequence of SEQ ID NO: 29, and the LCDR3 comprises the sequence of SEQ ID NO: 30; or

[0017] b) the HCDR1 comprises the sequence of SEQ ID NO: 31, the HCDR2 comprises the sequence of SEQ ID NO: 32 or SEQ ID NO: 37, and the HCDR3 comprises the sequence of SEQ ID NO: 33, the LCDR1 comprises the sequence of SEQ ID NO: 28, the LCDR2 comprises the sequence of SEQ ID NO: 29, and the LCDR3 comprises the sequence of SEQ ID NO: 30; or

[0018] c) the HCDR1 comprises the sequence of SEQ ID NO: 34, the HCDR2 comprises the sequence of SEQ ID NO: 35 or SEQ ID NO: 37, and the HCDR3 comprises the sequence of SEQ ID NO: 36, the LCDR1 comprises the sequence of SEQ ID NO: 28, the LCDR2 comprises the sequence of SEQ ID NO: 29, and the LCDR3 comprises the sequence of SEQ ID NO: 30.

[0019] In certain embodiments, in the anti-hVEGFR antibody or an antigen-binding fragment thereof provided herein, wherein the HCDR1 comprises an amino acid sequence of SEQ ID NO: 44, the HCDR2 comprises an amino acid sequence of SEQ ID NO: 45, the HCDR3 comprises an amino acid sequence of SEQ ID NO: 46, the LCDR1 comprises a sequence of SEQ ID NO: 47, the LCDR2 comprises a sequence of SEQ ID NO: 48, and the LCDR3 comprises a sequence of SEQ ID NO: 49.

[0020] In certain embodiments, in the antibody or an antigen-binding fragment thereof provided herein, wherein

[0021] a) the HCDR1 comprises the sequence of SEQ ID NO: 7, a HCDR2 comprises the sequence of SEQ ID NO: 8, the HCDR3 comprises the sequence of SEQ ID NO: 9; the LCDR1 comprises the sequence of SEQ ID NO: 10, the LCDR2 comprises the sequence of SEQ ID NO: 11, and the LCDR3 comprises the sequence of SEQ ID NO: 12; or

[0022] b) the HCDR1 comprises the sequence of SEQ ID NO: 13, the HCDR2 comprises the sequence of SEQ ID NO: 14, and the HCDR3 comprises the sequence of

SEQ ID NO: 15, the LCDR1 comprises the sequence of SEQ ID NO: 16, the LCDR2 comprises the sequence of SEQ ID NO: 17, and the LCDR3 comprises the sequence of SEQ ID NO: 18.

[0023] In certain embodiments, in the antibody or an antigen-binding fragment thereof provided herein, wherein

[0024] a) the HCDR1 comprises the sequence of SEQ ID NO: 1, the HCDR2 comprises the sequence of SEQ ID NO: 2, and the HCDR3 comprises the sequence of SEQ ID NO: 3, the LCDR1 comprises the sequence of SEQ ID NO: 4, the LCDR2 comprises the sequence of SEQ ID NO: 5, and the LCDR3 comprises the sequence of SEQ ID NO: 6; or

[0025] b) the HCDR1 comprises the sequence of SEQ ID NO: 19, the HCDR2 comprises the sequence of SEQ ID NO: 20, and the HCDR3 comprises the sequence of SEQ ID NO: 21, the LCDR1 comprises the sequence of SEQ ID NO: 22, the LCDR2 comprises the sequence of SEQ ID NO: 23, and the LCDR3 comprises the sequence of SEQ ID NO: 24.

[0026] In certain embodiments, the antibody or an antigen-binding fragment thereof provided herein further comprises one or more of heavy chain HFR1, HFR2, HFR3 and HFR4, and/or one or more of light chain LFR1, LFR2, LFR3 and LFR4, wherein:

[0027] the HFR1 comprises EVQLVESGGGLVKPGGSLXioLSCAASGFTFS (SEQ ID NO: 84) or a homologous sequence of at least 80% (or at least 85%, 90%, 95%) sequence identity thereof,

[0028] the HFR2 comprises WVRQX_{11}PGKRLEWVA (SEQ ID NO: 85) or a homologous sequence of at least 80% (or at least 90%) sequence identity thereof,

[0029] the HFR3 sequence comprises RFTISRDNAKNTLYLQMNSLX_{12}AEDTAVYYCAR (SEQ ID NO: 86) or a homologous sequence of at least 80% (or at least 85%, 90%, 95%) sequence identity thereof,

[0030] the HFR4 comprises WGX_{13}GTTLTVSS (SEQ ID NO: 87) or a homologous sequence of at least 80% sequence identity thereof,

[0031] the LFR1 comprises DIVITQ$X_{14}X_{15}$LSLPVTX_{16}GESVSISC (SEQ ID NO: 88) or a homologous sequence of at least 80% (or at least 85%, 90%, 95%) sequence identity thereof,

[0032] the LFR2 comprises WFLQRPGQSPQLLIY (SEQ ID NO: 89) or a homologous sequence of at least 80% (or at least 85%, 90%) sequence identity thereof,

[0033] the LFR3 comprises GVX_{17}DRFSGSGSGTDFTLKISRVEAEDVGX_{18}YYC (SEQ ID NO: 90) or a homologous sequence of at least 80% (or at least 85%, 90%, 95%) sequence identity thereof, and

[0034] the LFR4 comprises FGSGTKLEIK (SEQ ID NO: 91) or a homologous sequence of at least 80% (or at least 90%) sequence identity thereof, wherein X_{10} is R or K, X_{11} is A or T, X_{12} is R or K, X_{13} is Q or H, X_{14} is D or T, X_{15} is E or P, X_{16} is F or P, X_{17} is S or P, X_8 is V or I.

[0035] In certain embodiments, in the antibody or antigen-binding fragment thereof provided herein, wherein:

[0036] the HFR1 comprises a sequence selected from the group consisting of SEQ ID NOs: 64, 68 and 72,

[0037] the HFR2 comprises a sequence selected from the group consisting of SEQ ID NOs: 65, 69 and 73,

[0038] the HFR3 comprises the sequence selected from the group consisting of SEQ ID NOs: 66, 70 and 74,

[0039] the HFR4 comprises a sequence selected from the group consisting of SEQ ID NOs: 67, 71 and 75,

[0040] the LFR1 comprises the sequence selected from the group consisting of SEQ ID NOs: 76 and 80,

[0041] the LFR2 comprises a sequence selected from the group consisting of SEQ ID NO: 77 and 81,

[0042] the LFR3 comprises a sequence selected from the group consisting of SEQ ID NOs: 78 and 82, and

[0043] the LFR4 comprises a sequence selected from the group consisting of SEQ ID NOs: 79 and 83.

[0044] In certain embodiments, in the antibody or an antigen-binding fragment thereof provided herein, wherein the heavy chain variable region comprises a sequence selected from the group consisting of SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 98, and a homologous sequence thereof having at least 80% sequence identity yet retaining specific binding affinity to hVEGFR2.

[0045] In certain embodiments, in the antibody or an antigen-binding fragment thereof provided herein, wherein the light chain variable region comprises a sequence selected from the group consisting of SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 96, SEQ ID NO: 97, and a homologous sequence thereof having at least 80% sequence identity yet retaining specific binding affinity to hVEGFR2.

[0046] In certain embodiments, in the antibody or an antigen-binding fragment thereof provided herein, wherein:

[0047] a) a heavy chain variable region comprising the sequence of SEQ ID NO: 50 and a light chain variable region comprising the sequence of SEQ ID NO: 51;

[0048] b) the heavy chain variable region comprises a sequence of SEQ ID NO: 52 and the light chain variable region comprises a sequence of SEQ ID NO: 53;

[0049] c) the heavy chain variable region comprises a sequence of SEQ ID NO: 54 and the light chain variable region comprises a sequence of SEQ ID NO: 55;

[0050] d) the heavy chain variable region comprises a sequence of SEQ ID NO: 56 and the light chain variable region comprises a sequence of SEQ ID NO: 57;

[0051] e) the heavy chain variable region comprises a sequence of SEQ ID NO: 58 and the light chain variable region comprises a sequence of SEQ ID NO: 59;

[0052] f) the heavy chain variable region comprises a sequence of SEQ ID NO: 60 and the light chain variable region comprises a sequence of SEQ ID NO: 61;

[0053] g) the heavy chain variable region comprises a sequence of SEQ ID NO: 62 and the light chain variable region comprises a sequence of SEQ ID NO: 63; or

[0054] h) the heavy chain variable region comprises a sequence of SEQ ID NO: 93 or SEQ ID NO: 94 or SEQ ID NO: 98 and the light chain variable region comprises a sequence of SEQ ID NO: 96 or SEQ ID NO: 97.

[0055] In certain embodiments, the antibody or antigen-binding fragment thereof provided herein further comprises one or more amino acid residue substitutions or modifications yet retains specific binding affinity to hVEGFR2.

[0056] In certain embodiments, at least one of the substitutions or modifications is in one or more of the CDR sequences, and/or in one or more of the non-CDR regions of the VH or VL sequences.

[0057] In certain embodiments, the antibody or antigen-binding fragment provided herein further comprises an immunoglobulin constant region, optionally a constant region of human Ig, or optionally a constant region of human IgG.

[0058] In certain embodiments, the constant region comprises a constant region of human IgG1, IgG2, IgG3, or IgG4.

[0059] In certain embodiments, the constant region of human IgG1 comprises SEQ ID NO: 38, or a homologous sequence having at least 80% sequence identity thereof.

[0060] In certain embodiments, the antibody or an antigen-binding fragment thereof provided herein is humanized.

[0061] In certain embodiments, the antibody or antigen-binding fragment thereof provided herein is a diabody, a Fab, a Fab', a F(ab')2, a Fd, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a (dsFv)2, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), an scFv dimer (bivalent diabody), a multispecific antibody, a camelized single domain antibody, a nanobody, a domain antibody, or a bivalent domain antibody.

[0062] In certain embodiments, the antibody or antigen-binding fragment thereof provided herein is bispecific.

[0063] In certain embodiments, the antibody or antigen-binding fragment thereof provided herein is capable of specifically binding to a first and a second epitope of hVEGFR2, or capable of specifically binding to hVEGFR2 and a second antigen.

[0064] In certain embodiments, the second antigen is an immune related target, optionally selected from the group consisting of. PD-L1, PD-L2, PD-1, CLTA-4, TIM-3, LAG3, CD160, 2B4, TGFβ, VISTA, BTLA, TIGIT, LAIR1, OX40, CD2, CD27, ICAM-1, NKG2C, SLAMF7, NKp80, CD160, B7-H3, LFA-1, 1COS, 4-1BB, GITR, CD30, CD40, BAFFR, HVEM, CD7, LIGHT, IL-2, IL-15, CD3, CD16 and CD83.

[0065] In certain embodiments, the second antigen comprises a tumor antigen.

[0066] In certain embodiments, the tumor antigen is present in a VEGFR2-expressing cell.

[0067] In certain embodiments, the tumor antigen comprises claudin 18.2, CA-125, gangliosides G(D2), G(M2) and G(D3), CD20, CD52, CD33, Ep-CAM, CEA, bombesin-like peptides, PSA, HER2/neu, epidermal growth factor receptor (EGFR), erbB2, erbB3/HER3, erbB4, CD44v6, Ki-67, cancer-associated mucin, VEGF, VEGFRs (e.g., VEGFR3), estrogen receptors, Lewis-Y antigen, TGFβ1, IGF-1 receptor, EGFα, c-Kit receptor, transferrin receptor, IL-2R or CO17-1A.

[0068] In certain embodiments, the antibody or antigen-binding fragment thereof provided herein is linked to one or more conjugate moieties.

[0069] In certain embodiments, the conjugate moiety comprises a clearance-modifying agent, a chemotherapeutic agent, a toxin, a radioactive isotope, a lanthanide, a luminescent label, a fluorescent label, an enzyme-substrate label, a DNA-alkylators, a topoisomerase inhibitor, a tubulin-binder, or other anticancer drugs.

[0070] In another aspect, the present disclosure also provides an antibody or an antigen-binding fragment thereof that competes for binding to hVEGFR2 with the antibody or antigen-binding fragment provided herein.

[0071] In one aspect, the present disclosure provides a pharmaceutical composition comprising the antibody or antigen-binding fragment provided herein, and one or more pharmaceutically acceptable carriers.

[0072] In certain embodiments, the pharmaceutical composition provided herein further comprises a second therapeutic agent.

[0073] In certain embodiments, the second therapeutic agent comprises an anti-cancer therapy, optionally the anti-cancer therapy is selected from a chemotherapeutic agent, radiation therapy, an immunotherapy agent, anti-angiogenesis agent (e.g. antagonist of a VEGFR such as VEGFR-1, and VEGFR-3), an EGFR antagonist, an PDGFR antagonist, an IGFR antagonist, an NGFR antagonist, an FGFR antagonist, a targeted therapy agent (e.g. HER2 antibody, claudin 18.2 antibody), a cellular therapy agent, a gene therapy agent, a hormonal therapy agent, cytokines, palliative care, surgery for the treatment of cancer (e.g., tumorectomy), one or more anti-emetics, treatments for complications arising from chemotherapy, or a diet supplement for cancer patients (e.g. indole-3-carbinol).

[0074] In one aspect, the present disclosure provides an isolated polynucleotide encoding the antibody or an antigen-binding fragment thereof provided herein.

[0075] In one aspect, the present disclosure provides a vector comprising the isolated polynucleotide provided herein.

[0076] In one aspect, the present disclosure provides a host cell comprising the vector provided herein.

[0077] In one aspect, the present disclosure provides a method of expressing the antibody or antigen-binding fragment thereof provided herein, comprising culturing the host cell provided herein under the condition at which the vector provided herein is expressed.

[0078] In one aspect, the present disclosure provides a method of treating, reducing the severity of and/or slowing the progression of a VEGFR2-related disease or condition in a subject, comprising administering to the subject a therapeutically effective amount of the antibody or antigen-binding fragment thereof provided herein, or the pharmaceutical composition provided herein.

[0079] In certain embodiments, the VEGFR2-related disease or condition is a tumor or an angiogenic disease.

[0080] In certain embodiments, the tumor produces VEGF (e.g., VEGF-A) and/or is sensitive to VEGF (e.g., VEGF-A) present in its microenvironment.

[0081] In certain embodiments, the tumor is a solid tumor or non-solid tumor.

[0082] In certain embodiments, the solid tumor is selected from the group consisting of breast carcinoma, lung carcinoma, colorectal carcinoma, pancreatic carcinoma, glioma and lymphoma, head and neck tumors, neuroendocrine tumors, colorectal tumors, prostate tumors, breast tumors, lung tumors, such as small cell and non-small cell lung tumors, pancreatic tumors, thyroid tumors, ovarian tumors, cervical tumors, kidney tumors, brain tumors, liver tumors, Kaposi's sarcoma, CNS neoplasms, neuroblastomas, capil-

lary hemangioblastomas, meningiomas, cerebral metastases, melanoma, gastrointestinal and renal carcinomas and sarcomas (e.g., gastric cancer), rhabdomyosarcoma, glioblastoma, preferably glioblastoma multiforme, leiomyosarcoma, squamous cell carcinoma, basal cell carcinoma and skin cancers that can be treated by suppressing the growth of malignant keratinocytes, such as human malignant keratinocytes.

[0083] In certain embodiments, the tumor is selected from the group consisting of gastric cancer, non-small cell lung cancer, such as large cell lung cancer.

[0084] In certain embodiments, the non-solid tumor is selected from the group consisting of leukemia, multiple myeloma and lymphoma, for example, acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), erythrocytic leukemia ormonocytic leukemia, Hodgkin's and non-Hodgkin's lymphoma.

[0085] In certain embodiments, the angiogenic disease is selected from the group consisting of atherosclerosis, rheumatoid arthritis (RA), neovascular glaucoma, proliferative retinopathy including proliferative diabetic retinopathy, macular degeneration, hemangiomas, angiofibromas, psoriasis, retinopathy of prematurity (e.g., retrolental fibroplastic), corneal graft rejection, insulin-dependent diabetes mellitus, multiple sclerosis, myasthenia gravis, Chron's disease, autoimmune nephritis, primary biliary cirrhosis, acute pancreatitis, allograph rejection, allergic inflammation, contact dermatitis and delayed hypersensitivity reactions, inflammatory bowel disease, septic shock, osteoporosis, osteoarthritis, cognition defects induced by neuronal inflammation, Osler-Weber syndrome, restinosis, and fungal, parasitic and viral infections, such as cytomegaloviral infections.

[0086] In certain embodiments, the subject is human.

[0087] In certain embodiments, the administration is via oral, nasal, intravenous, subcutaneous, sublingual, intratumoral, or intramuscular administration.

[0088] In certain embodiments, the method provided herein further comprises administering a therapeutically effective amount of a second therapeutic agent.

[0089] In certain embodiments, the second therapeutic agent comprises an anti-cancer therapy, optionally the anti-cancer therapy is selected from a chemotherapeutic agent, radiation therapy, an immunotherapy agent, anti-angiogenesis agent (e.g. antagonist of a VEGFR such as VEGFR-1, VEGFR-2, and VEGFR-3), an EGFR antagonist, an PDGFR antagonist, an IGFR antagonist, an NGFR antagonist, an FGFR antagonist, a targeted therapy agent, a cellular therapy agent, a gene therapy agent, a hormonal therapy agent, cytokines, palliative care, surgery for the treatment of cancer (e.g., tumorectomy), one or more anti-emetics, treatments for complications arising from chemotherapy, or a diet supplement for cancer patients (e.g. indole-3-carbinol).

[0090] In one aspect, the present disclosure provides a kit comprising an antibody or an antigen-binding fragment thereof provided herein.

[0091] In one aspect, the present disclosure provides a method of detecting presence or amount of VEGFR2 in a sample, comprising contacting the sample with the antibody or antigen-binding fragment thereof provided herein, and determining the presence or the amount of VEGFR2 in the sample.

[0092] In one aspect, the present disclosure provides use of the antibody or antigen-binding fragment thereof provided herein in the manufacture of a medicament for treating, reducing the severity of and/or slowing the progression of a VEGFR2-related disease or condition in a subject.

[0093] In certain embodiments, the VEGFR2-related disease or condition is a tumor or an angiogenic disease.

[0094] In certain embodiments, the tumor produces VEGF (e.g., VEGF-A) and/or is sensitive to VEGF (e.g., VEGF-A) present in its microenvironment.

[0095] In certain embodiments, the tumor is a solid tumor or non-solid tumor.

[0096] In certain embodiments, the angiogenic disease is selected from the group consisting of atherosclerosis, rheumatoid arthritis (RA), neovascular glaucoma, proliferative retinopathy including proliferative diabetic retinopathy, macular degeneration, hemangiomas, angiofibromas, psoriasis, retinopathy of prematurity (e.g., retrolental fibroplastic), corneal graft rejection, insulin-dependent diabetes mellitus, multiple sclerosis, myasthenia gravis, Chron's disease, autoimmune nephritis, primary biliary cirrhosis, acute pancreatitis, allograph rejection, allergic inflammation, contact dermatitis and delayed hypersensitivity reactions, inflammatory bowel disease, septic shock, osteoporosis, osteoarthritis, cognition defects induced by neuronal inflammation, Osler-Weber syndrome, restinosis, and fungal, parasitic and viral infections, such as cytomegaloviral infections.

BRIEF DESCRIPTION OF FIGURES

[0097] FIG. **1**A and FIG. **1**B show the dose-dependent binding of purified hybridoma antibodies #002, #003, #006, #018, #042, #048, and #054 to human VEGFR2 (hVEGFR2).

[0098] FIG. **2** shows the dose-dependent blocking activities of purified hybridoma antibodies for human VEGF-A (hVEGF-A) binding to hVEGFR2.

[0099] FIG. **3** shows the binding of hybridoma antibodies to HUVEC cells.

[0100] FIG. **4**A and FIG. **4**B show chimeric antibodies that bind to hVEGFR2 and rhesus VEGFR2.

[0101] FIG. **5** shows that the chimeric antibodies blocked the VEGF-A/VEGFR2 interaction.

[0102] FIG. **6** shows the binding of chimeric antibodies to HUVEC cells.

[0103] FIG. **7**A and FIG. **7**B show epitope binning using Biotin-mAB002 by competition assay.

[0104] FIG. **8**A, FIG. **8**B and FIG. **8**C show the dose-dependent competition assay results.

[0105] FIG. **9**A, FIG. **9**B and FIG. **9**C show that the chimeric antibodies inhibit phosphorylation of VEGFR2 induced by VEGF-A.

[0106] FIG. **10**A and FIG. **10**B show humanized antibodies produced in 293T cells binding to human VEGFR2-his by ELISA.

[0107] FIG. **11**A and FIG. **11**B show humanized antibodies produced in CHO cells binding to human VEGFR2-his and rhesus by ELISA.

[0108] FIG. **12** shows humanized antibodies that bind specifically to VEGFR2.

[0109] FIG. **13**A and FIG. **13**B show humanized antibodies blocking the binding of VEGF-A to human VEGFR2 or rhesus VEGFR2.

[0110] FIG. **14**A, FIG. **14**B and FIG. **14**C show humanized antibodies blocking VEGF-A, C or D binding to VEGFR2.

[0111] FIG. **15** shows humanized antibodies binding to HUVEC cells.

[0112] FIG. **16** shows that humanized antibody 054 inhibits VEGF-A induced VEGFR2 phosphorylation.

[0113] FIG. **17** shows humanized antibodies blocking VEGF-A mediated cell proliferation.

[0114] FIG. **18** shows the survival curve of HL-60 engraftment on NOD/SCID mice treated with a benchmark antibody 1121B, chimeric 054 (54-C), chimeric 002 (2-C) or saline.

[0115] FIG. **19** shows ELISA binding of Chimeric-DC101 to mouse VEGFR2.

[0116] FIG. **20** shows blockade of mouse VEGFR2 binding to human VEGF165 (a splice variant of VEGF-A) by Chimeric-DC101.

[0117] FIG. **21** shows tumor growth curve of MKN45 xenograft tumor on nude mice (mean±S.E.M., n=10).

[0118] FIG. **22**. shows tumor growth curve of H460 xenograft tumor on nude mice (mean±S.E.M., n=10)

[0119] FIG. **23** shows tumor growth curve of H1975 xenograft tumor on nude mice (mean±S.E.M., n=10)

DETAILED DESCRIPTION OF THE INVENTION

[0120] The following description of the disclosure is merely intended to illustrate various embodiments of the disclosure. As such, the specific modifications discussed are not to be construed as limitations on the scope of the disclosure. It will be apparent to one skilled in the art that various equivalents, changes, and modifications may be made without departing from the scope of the disclosure, and it is understood that such equivalent embodiments are to be included herein. All references cited herein, including publications, patents and patent applications are incorporated herein by reference in their entirety.

Definitions

[0121] As used herein, the term "a," "an," "the" and similar terms used in the context of the present invention (especially in the context of the claims) are to be construed to cover both the singular and plural unless otherwise indicated herein or clearly contradicted by the context.

[0122] The term "antibody" as used herein includes any immunoglobulin, monoclonal antibody, polyclonal antibody, multivalent antibody, bivalent antibody, monovalent antibody, multispecific antibody, or bispecific antibody that binds to a specific antigen. A native intact antibody comprises two heavy (H) chains and two light (L) chains. Mammalian heavy chains are classified as alpha, delta, epsilon, gamma, and mu, each heavy chain consists of a variable region (V_H) and a first, second, and third constant region (C_{H1}, C_{H2}, C_{H3}, respectively); mammalian light chains are classified as X or x, while each light chain consists of a variable region (V_L) and a constant region. The antibody has a "Y" shape, with the stem of the Y consisting of the second and third constant regions of two heavy chains bound together via disulfide bonding. Each arm of the Y includes the variable region and first constant region of a single heavy chain bound to the variable and constant regions of a single light chain. The variable regions of the light and heavy chains are responsible for antigen binding. The variable regions in both chains generally contain three highly variable loops called the complementarity determining regions (CDRs) (light chain CDRs including LCDR1, LCDR2, and LCDR3, heavy chain CDRs including HCDR1, HCDR2, HCDR3). CDR boundaries for the antibodies and antigen-binding domains disclosed herein may be defined or identified by the conventions of Kabat, IMGT, AbM, Chothia, or Al-Lazikani (Al-Lazikani, B., Chothia, C., Lesk, A. M., J. Mol. Biol., 273(4), 927 (1997); Chothia, C. et al., J Mol Biol. Dec. 5; 186(3):651-63 (1985); Chothia, C. and Lesk, A. M., J.Mol.Biol., 196,901 (1987); N. R. Whitelegg et al, Protein Engineering, v13(12), 819-824 (2000); Chothia, C. et al., Nature. Dec. 21-28; 342(6252):877-83 (1989); Kabat E.A. et al., National Institutes of Health, Bethesda, Md. (1991); Marie-Paule Lefranc et al, Developmental and Comparative Immunology, 27: 55-77 (2003); Marie-Paule Lefranc et al, Immunome Research, 1(3), (2005); Marie-Paule Lefranc, Molecular Biology of B cells (second edition), chapter 26, 481-514, (2015)). The three CDRs are interposed between flanking stretches known as framework regions (FRs), which are more highly conserved than the CDRs and form a scaffold to support the hypervariable loops. The constant regions of the heavy and light chains are not involved in antigen-binding, but exhibit various effector functions. Antibodies are assigned to classes based on the amino acid sequence of the constant region of their heavy chain. The five major classes or isotypes of antibodies are IgA, IgD, IgE, IgG, and IgM, which are characterized by the presence of alpha, delta, epsilon, gamma, and mu heavy chains, respectively. Several of the major antibody classes are divided into subclasses such as IgG1 (gamma1 heavy chain), IgG2 (gamma2 heavy chain), IgG3 (gamma3 heavy chain), IgG4 (gamma4 heavy chain), IgA1 (alpha1 heavy chain), or IgA2 (alpha2 heavy chain). In certain embodiments, the antibody provided herein encompasses any antigen-binding fragments thereof.

[0123] As used herein, the term "antigen-binding fragment" refers to an antibody fragment formed from a fragment of an antibody comprising one or more CDRs, or any other antibody portion that binds to an antigen but does not comprise an intact native antibody structure. Examples of antigen-binding fragment include, without limitation, a diabody, a Fab, a Fab', a $F(ab')_2$, a Fd, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), an scFv dimer (bivalent diabody), a multispecific antibody, a camelized single domain antibody, a nanobody, a domain antibody, and a bivalent domain antibody. An antigen-binding fragment is capable of binding to the same antigen to which the parent antibody binds. In certain embodiments, an antigen-binding fragment may comprise one or more CDRs from a particular human antibody.

[0124] "Fab" with regard to an antibody refers to a monovalent antigen-binding fragment of the antibody consisting of a single light chain (both variable and constant regions) bound to the variable region and first constant region of a single heavy chain by a disulfide bond. Fab can be obtained by papain digestion of an antibody at the residues proximal to the N-terminus of the disulfide bond between the heavy chains of the hinge region.

[0125] "Fab" refers to a Fab fragment that includes a portion of the hinge region, which can be obtained by pepsin digestion of an antibody at the residues proximal to the C-terminus of the disulfide bond between the heavy chains

of the hinge region and thus is different from Fab in a small number of residues (including one or more cysteines) in the hinge region.

[0126] "F(ab')2" refers to a dimer of Fab' that comprises two light chains and part of two heavy chains.

[0127] "Fc" with regard to an antibody refers to that portion of the antibody consisting of the second and third constant regions of a first heavy chain bound to the second and third constant regions of a second heavy chain via disulfide bond. IgG and IgM Fc regions contain three heavy chain constant regions (second, third and fourth heavy chain constant regions in each chain). It can be obtained by papain digestion of an antibody. The Fc portion of the antibody is responsible for various effector functions such as ADCC, ADCP and CDC, but does not function in antigen binding.

[0128] "Fv" with regard to an antibody refers to the smallest fragment of the antibody to bear the complete antigen binding site. A Fv fragment consists of the variable region of a single light chain bound to the variable region of a single heavy chain. A "dsFv" refers to a disulfide-stabilized Fv fragment that the linkage between the variable region of a single light chain and the variable region of a single heavy chain is a disulfide bond.

[0129] "Single-chain Fv antibody" or "scFv" refers to an engineered antibody consisting of a light chain variable region and a heavy chain variable region connected to one another directly or via a peptide linker sequence (Huston J S et al. *Proc Natl Acad Sci USA,* 85:5879(1988)). A "scFv dimer" refers to a single chain comprising two heavy chain variable regions and two light chain variable regions with a linker. In certain embodiments, an "scFv dimer" is a bivalent diabody or bivalent ScFv (BsFv) comprising V_H-V_L (linked by a peptide linker) dimerized with another V_H-$V_L$moiety such that V_H's of one moiety coordinate with the V_L's of the other moiety and form two binding sites which can target the same antigens (or eptipoes) or different antigens (or eptipoes). In other embodiments, a "scFv dimer" is a bispecific diabody comprising V_{H1}—V_{L2} (linked by a peptide linker) associated with V_{L1}-V_{H2} (also linked by a peptide linker) such that V_{H1} and V_{L1} coordinate and V_{H2} and V_{L2} coordinate and each coordinated pair has a different antigen specificity.

[0130] "Single-chain Fv-Fc antibody" or "scFv-Fc" refers to an engineered antibody consisting of a scFv connected to the Fc region of an antibody.

[0131] "Camelized single domain antibody," "heavy chain antibody," "nanobody" or "HCAb" refers to an antibody that contains two V_H domains and no light chains (Riechmann L. and Muyldermans S., *J Immunol Methods*. Dec. 10; 231(1-2):25-38 (1999); Muyldermans S., J Biotechnol. Jun.; 74(4): 277-302 (2001); WO94/04678; WO94/25591; U.S. Pat. No. 6,005,079). Heavy chain antibodies were originally obtained from *Camelidae* (camels, dromedaries, and llamas). Although devoid of light chains, camelized antibodies have an authentic antigen-binding repertoire (Hamers-Casterman C. et al., *Nature*. Jun. 3; 363(6428):446-8 (1993); Nguyen VK. et al. "Heavy-chain antibodies in Camelidae; a case of evolutionary innovation," Immunogenetics. Apr.; 54(1):39-47 (2002); Nguyen V K. et al. *Immunology*. May; 109(1): 93-101 (2003)). The variable domain of a heavy chain antibody (VHH domain) represents the smallest known antigen-binding unit generated by adaptive immune responses (Koch-Nolte F. et al., *FASEB J*. Nov.; 21(13): 3490-8. Epub 2007 Jun. 15 (2007)). "Diabodies" include small antibody fragments with two antigen-binding sites, wherein the fragments comprise a V_H domain connected to a V_L domain in a single polypeptide chain (V_H-V_L or V_L-V_H) (see, e.g., Holliger P. et al., *Proc Natl Acad Sci USA*. Jul. 15; 90(14):6444-8 (1993); EP404097; WO93/11161). The two domains on the same chain cannot be paired, because the linker is too short, thus, the domains are forced to pair with the complementary domains of another chain, thereby creating two antigen-binding sites. The antigen-binding sites may target the same of different antigens (or epitopes).

[0132] A "domain antibody" refers to an antibody fragment containing only the variable region of a heavy chain or the variable region of a light chain. In certain embodiments, two or more $V_H$domains are covalently joined with a peptide linker to form a bivalent or multivalent domain antibody. The two V_H domains of a bivalent domain antibody may target the same or different antigens.

[0133] In certain embodiments, a "$(dsFv)_2$" comprises three peptide chains: two V_H moieties linked by a peptide linker and bound by disulfide bridges to two V_L moieties.

[0134] In certain embodiments, a "bispecific ds diabody" comprises V_{H1}—V_{L2} (linked by a peptide linker) bound to V_{L1}-V_{H2} (also linked by a peptide linker) via a disulfide bridge between V_{H1} and V_L1.

[0135] In certain embodiments, a "bispecific dsFv" or "dsFv-dsFv" comprises three peptide chains: a V_H1-V_{H2} moiety wherein the heavy chains are bound by a peptide linker (e.g., a long flexible linker) and paired via disulfide bridges to V_{L1} and V_{L2} moieties, respectively. Each disulfide paired heavy and light chain has a different antigen specificity.

[0136] The term "humanized" as used herein means that the antibody or antigen-binding fragment comprises CDRs derived from non-human animals, FR regions derived from human, and when applicable, constant regions derived from human. In certain embodiments, the amino acid residues of the variable region framework of the humanized hVEGFR2 antibody are substituted for sequence optimization. In certain embodiments, the variable region framework sequences of the humanized hVEGFR2 antibody chain are at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% identical to the corresponding human variable region framework sequences.

[0137] The term "chimeric" as used herein refers to an antibody or antigen-binding fragment that has a portion of heavy and/or light chain derived from one species, and the rest of the heavy and/or light chain derived from a different species. In an illustrative example, a chimeric antibody may comprise a constant region derived from human and a variable region derived from a non-human species, such as from mouse.

[0138] The term "germline sequence" refers to the nucleic acid sequence encoding a variable region amino acid sequence or subsequence that shares the highest determined amino acid sequence identity with a reference variable region amino acid sequence or subsequence in comparison to all other known variable region amino acid sequences encoded by germline immunoglobulin variable region sequences. The germline sequence can also refer to the variable region amino acid sequence or subsequence with the highest amino acid sequence identity with a reference variable region amino acid sequence or subsequence in comparison to all other evaluated variable region amino acid

sequences. The germline sequence can be framework regions only, complementarity determining regions only, framework and complementarity determining regions, a variable segment (as defined above), or other combinations of sequences or subsequences that comprise a variable region. Sequence identity can be determined using the methods described herein, for example, aligning two sequences using BLAST, ALIGN, or another alignment algorithm known in the art. The germline nucleic acid or amino acid sequence can have at least about 90%, 91, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the reference variable region nucleic acid or amino acid sequence. Germline sequences can be determined, for example, through the publicly available international ImMunoGeneTics database (IMGT) and V-base.

[0139] "Anti-hVEGFR2 antibody" or "an antibody against hVEGFR2" as used herein refers to an antibody that is capable of specific binding to human VEGFR2 with a sufficient affinity, for example, to provide for diagnostic and/or therapeutic use.

[0140] The term "affinity" as used herein refers to the strength of non-covalent interaction between an immunoglobulin molecule (i.e. antibody) or fragment thereof and an antigen.

[0141] The term "specific binding" or "specifically binds" as used herein refers to a non-random binding reaction between two molecules, such as for example between an antibody and an antigen.

[0142] In certain embodiments, the antibodies or antigen-binding fragments provided herein specifically bind to hVEGFR2 with a binding affinity (K_D) of $\leq 10^{-6}$ M (e.g., $\leq 5\times10^{-7}$ M, $\leq 2\times10^{-7}$ M, $\leq 10^{-7}$ M, $\leq 5\times10^{-8}$ M, $\leq 2\times10^{-8}$ M, $\leq 10^{-8}$ M, $\leq 5\times10^{-9}$ M, $\leq 4\times10^{-9}$ M, $\leq 3\times10^{-9}$ M,$\leq 2\times10^{-9}$ M, or $\leq 10^{-9}$ M. K_D used herein refers to the ratio of the dissociation rate to the association rate (k_{off}/k_{on}), which may be determined by using any conventional method known in the art, including but are not limited to surface plasmon resonance method, microscale thermophoresis method, HPLC-MS method and flow cytometry (such as FACS) method. In certain embodiments, the K_D value can be appropriately determined by using flow cytometry method. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (see, e.g., Harlow & Lane, Using Antibodies, A Laboratory Manual (1998), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Typically a specific or selective binding reaction will produce a signal at least twice over the background signal and more typically at least 10 to 100 times over the background.

[0143] "Percent (%) sequence identity" with respect to amino acid sequence (or nucleic acid sequence) is defined as the percentage of amino acid (or nucleic acid) residues in a candidate sequence that are identical to the amino acid (or nucleic acid) residues in a reference sequence, after aligning the sequences and, if necessary, introducing gaps, to achieve the maximum correspondence. Alignment for purposes of determining percent amino acid (or nucleic acid) sequence identity can be achieved, for example, using publicly available tools such as BLASTN, BLASTp (available on the website of U.S. National Center for Biotechnology Information (NCBI), see also, Altschul S.F. et al, J. Mol. Biol., 215:403-410 (1990); Stephen F. et al, Nucleic Acids Res., 25:3389-3402 (1997)), ClustalW2 (available on the website of European Bioinformatics Institute, see also, Higgins D. G. et al, Methods in Enzymology, 266:383-402 (1996); Larkin M.A. et al, Bioinformatics (Oxford, England), 23(21): 2947-8 (2007)), and ALIGN or Megalign (DNASTAR) software. Those skilled in the art may use the default parameters provided by the tool, or may customize the parameters as appropriate for the alignment, such as for example, by selecting a suitable algorithm. In certain embodiments, the non-identical residue positions may differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. See, e.g., Pearson (1994) Methods Mol. Biol. 24: 307-331, which is herein incorporated by reference.

[0144] As used herein, a "homologue sequence" and "homologous sequence" are used interchangeable and refer to polynucleotide sequences (or its complementary strand) or amino acid sequences that have sequences identity of at least 80% (e.g. at least 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%) to another sequences when optionally aligned.

[0145] An "isolated" substance has been altered by the hand of man from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide is "isolated" if it has been sufficiently separated from the coexisting materials of its natural state so as to exist in a substantially pure state. An isolated "nucleic acid" or "polynucleotide" are used interchangeably and refer to the sequence of an isolated nucleic acid molecule. In certain embodiments, an "isolated antibody or antigen-binding fragment thereof" refers to the antibody or antigen-binding fragments having a purity of at least 60%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% as determined by electrophoretic methods (such as SDS-PAGE, isoelectric focusing, capillary electrophoresis), or chromatographic methods (such as ion exchange chromatography or reverse phase HPLC).

[0146] The ability to "block(s) binding" as used herein refers to the ability of an antibody or antigen-binding fragment to inhibit the binding interaction between two molecules (e.g. VEGR-A and hVEGFR2) to any detectable degree. In certain embodiments, an antibody or antigen-binding fragment that blocks binding between two molecules (e.g. VEGR-A and hVEGFR2) inhibits the binding interaction between the two molecules by at least 50%. In certain embodiments, this inhibition may be greater than 60%, greater than 70%, greater than 80%, or greater than 90%.

[0147] The term "antibody drug conjugate" as used herein refers to the linkage of an antibody or an antigen binding fragment thereof with another agent, such as a chemotherapeutic agent, a toxin, an immunotherapeutic agent, an imaging probe, and the like. The linkage can be covalent bonds, or non-covalent interactions such as through electrostatic forces. Various linkers, known in the art, can be employed in order to form the antibody drug conjugate. Additionally, the antibody drug conjugate can be provided in the form of a fusion protein that may be expressed from a polynucleotide encoding the conjugate. As used herein, "fusion protein" refers to proteins created through the joining of two or more genes or gene fragments which originally coded for separate proteins (including peptides and polypeptides). Translation of the fusion gene results in a single protein with functional properties derived from each of the original proteins.

[0148] The term "subject" includes human and non-human animals. Non-human animals include all vertebrates, e.g., mammals and non-mammals, such as non-human primates, mouse, rat, cat, rabbit, sheep, dog, cow, chickens, amphibians, and reptiles. Except when noted, the terms "patient" or "subject" are used herein interchangeably.

[0149] "Effector functions" or "antibody effector functions" as used herein refer to biological activities attributable to the binding of Fc region of an antibody to its effectors such as C1 complex and Fc receptor. Exemplary effector functions include: complement dependent cytotoxicity (CDC) induced by interaction of antibodies and C1q on the C1 complex; antibody-dependent cell-mediated cytotoxicity (ADCC) induced by binding of Fc region of an antibody to Fc receptor on an effector cell; and antibody dependent cell mediated phagocytosis (ADCP), where nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause phagocytosis of the target cell. Effector functions include both those that operate after the binding of an antigen and those that operate independent of antigen binding.

[0150] "Treating" or "treatment" of a condition as used herein includes preventing or alleviating a condition, slowing the onset or rate of development of a condition, reducing the risk of developing a condition, preventing or delaying the development of symptoms associated with a condition, reducing or ending symptoms associated with a condition, generating a complete or partial regression of a condition, curing a condition, or some combination thereof.

[0151] The term "vector" as used herein refers to a vehicle into which a genetic element may be operably inserted so as to bring about the expression of that genetic element, such as to produce the protein, RNA or DNA encoded by the genetic element, or to replicate the genetic element. A vector may be used to transform, transduce, or transfect a host cell so as to bring about expression of the genetic element it carries within the host cell. Examples of vectors include plasmids, phagemids, cosmids, artificial chromosomes such as yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or P1-derived artificial chromosome (PAC), bacteriophages such as lambda phage or M13 phage, and animal viruses. A vector may contain a variety of elements for controlling expression, including promoter sequences, transcription initiation sequences, enhancer sequences, selectable elements, and reporter genes. In addition, the vector may contain an origin of replication. A vector may also include materials to aid in its entry into the cell, including but not limited to a viral particle, a liposome, or a protein coating. A vector can be an expression vector or a cloning vector. The present disclosure provides vectors (e.g. expression vectors) containing the nucleic acid sequence provided herein encoding the antibody or antigen-binding fragment thereof, at least one promoter (e.g. SV40, CMV, EF-1α) operably linked to the nucleic acid sequence, and at least one selection marker.

[0152] The "host cell" as used herein refers to a cell into which an exogenous polynucleotide and/or a vector has been introduced.

[0153] The term "VEGFR2", used interchangeably with the terms "VEGF receptor 2", "kinase insert domain-containing receptor (KDR)", "CD309", or "fetal liver kinase 1 (FLK1)" is one type of VEGF receptors (VEGFRs), which is a type III receptor tyrosine kinase characterized by having, typically, 5 or 7 immunoglobulin-like loops in their amino-terminal extracellular receptor ligand-binding domains, as well as a transmembrane region and a carboxy-terminal intracellular catalytic domain interrupted by an insertion of hydrophilic interkinase sequences of variable lengths called the kinase insert domain (Kaipainen et al., *J. Exp. Med.,* 178:2077-88 (1993); Terman et al., *Oncogene,* 6:1677-83 (1991)). Other types of VEGFRs include fins-like tyrosine kinase receptor (fit-1) or VEGFR1, and VEGFR3 (fit-4) (Shibuya et al., Oncogene, 5:519-24 (1990)). The amino-terminal extracellular receptor ligand-binding domains are responsible for the affinity between the ligand (e.g., vascular endothelial growth factor A (VEGF-A)) and the domains. The carboxy-terminal intracellular catalytic domain is responsible for the initiation of the signaling pathway involved in, for example, cell growth. Expression of VEGFRs can be found on endothelial cells during, e.g., embryogenesis and tumor formation (Millauer, B. et al. Cell, 72:835-46 (1993); Plate, K. et al. (1993)). Expression of VEGFRs can also be found on non-endothelial cells, such as tumor cells, especially tumor cells producing VEGF, e.g., leukemic cells, (Fielder et al., Blood 89:1870-5 (1997) and Bellamy et al., Cancer Res. 59728-33 (1999).).

Human VEGFR2 (hVEGFR2) used herein comprises an amino acid sequence that can be accessed via the accession number AAI31823.1 in the NCBI database or an amino acid sequence of SEQ ID NO: 39

(MQSKVLLAVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTIKANTTLQITCRGQRDLDWLWPNNQ
SGSEQRVEVTECSDGLFCKTLTIPKVIGNDTGAYKCFYRETDLASVIYVYVQDYRSPFIASVSDQHG
VVYITENKNKTVVIPCLGSISNLNVSLCARYPEKRFVPDGNRISWDSKKGFTIPSYMISYAGMVFCE
AKINDESYQSIMYIVVVVGYRIYDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDENWEYPSSKHQ
HKKLVNRDLKTQSGSEMKKELSTLTIDGITRSDQGLYTCAASSGLMTKKNSTFVRVHEKPFVAFGSG
MESLVEATVGERVRIPAKYLGYPPPEIKWYKNGIPLESNHTIKAGHVLTIMEVSERDTGNYTVILTN
PISKEKQSHVVSLVVYVPPQIGEKSLISPVDSYQYGTTQTLTCTVYAIPPPHHIHWYWQLEEECANE
PSHAVSVTNPYPCEEWRSVEDFQGGNKIEVNKNQFALIEGKNKTVSTLVIQAANVSALYKCEAVNKV
GRGERVISFHVTRGPEITLQPDMQPTEQESVSLWCTADRSTFENLTWYKLGPQPLPIHVGELPTPVC
KNLDTLWKLNATMESNSTNDILIMELKNASLQDQGDYVCLAQDRKTKKRHCVVRQLTVLERVAPTIT

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GNLENQTTSIGESIEVSCTASGNPPPQIMWFKDNETLVEDSGIVLKDGNRNLTIRRVRKEDEGLYTC
QACSVLGCAKVEAFFIIEGAQEKTNLEIIILVGTAVIAMFFWLLLVIILRTVKRANGGELKTGYLSI
VMDPDELPLDEHCERLPYDASKWEFPRDRLKLGKPLGRGAFGQVIEADAFGIDKTATCRTVAVKMLK
EGATHSEHRALMSELKILIHIGHHLNVVNLLGACTKPGGPLMVIVEFCKFGNLSTYLRSKRNEFVPY
KTKGARFRQGKDYVGAIPVDLKRRLDSITSSQSSASSGFVEEKSLSDVEEEEAPEDLYKDELTLEHL
ICYSFQVAKGMEFLASRKCIHRDLAARNILLSEKNVVKICDFGLARDIYKDPDYVRKGDARLPLKWM
APETIFDRVYTIQSDVWSFGVLLWEIFSLGASPYPGVKIDEEFCRRLKEGTRMRAPDYTTPEMYQTM
LDCWHGEPSQRPTFSELVEHLGNLLQANAQQDGKDYIVLPISETLSMEEDSGLSLPTSPVSCMEEEE
VCDPKFHYDNTAGISQYLQNSKRKSRPVSVKTFEDIPLEEPEVKVIPDDNQTDSGMVLASEELKTLE
DRTKLSPSFGGMVPSKSRESVASEGSNQTSGYQSGYHSDDTDTTVYSSEEAELLKLIEIGVQTGSTA
QILQPDSGTTLSSPPV).

Mouse VEGFR2 used herein comprises an amino acid sequence that can be accessed via the accession number P35918.1 in the NCBI database or an amino acid sequence of SEQ ID NO: 100
(MESKALLAVALWFCVETRAASVGLPGDFLHPPKLSTQKDILTILANTTLQITCRGQRDLDWLWPNAQ
RDSEERVLVTECGGGDSIFCKTLTIPRVVGNDTGAYKCSYRDVDIASTVYVYVRDYRSPFIASVSDQ
HGIVYITENKNKTVVIPCRGSISNLNVSLCARYPEKRFVPDGNRISWDSEIGFTLPSYMISYAGMVE
CEAKINDETYQSIMYIVVVVGYRIYDVILSPPHEIELSAGEKLVLNCTARTELNVGLDFTWHSPPSK
SHHKKIVNRDVKPFPGTVAKMFLSTLTIESVTKSDQGEYTCVASSGRMIKRNRTFVRVHTKPFIAFG
SGMKSLVEATVGSQVRIPVKYLSYPAPDIKWYRNGRPIESNYTMIVGDELTIMEVTERDAGNYTVIL
TNPISMEKQSHMVSLVVNVPPQIGEKALISPMDSYQYGTMQTLTCTVYANPPLHHIQWYWQLEEACS
YRPGQTSPYACKEWRHVEDFQGGNKIEVTKNQYALIEGKNKTVSTLVIQAANVSALYKCEAINKAGR
GERVISFHVIRGPEITVQPAAQPTEQESVSLLCTADRNTFENLTWYKLGSQATSVHMGESLTPVCKN
LDALWKLNGTMFSNSINDILIVAFQNASLQDQGDYVCSAQDKKTKKRHCLVKQLIILERMAPMITGN
LENQTTTIGETIEVTCPASGNPTPHITWFKDNETLVEDSGIVLRDGNRNLTIRRVRKEDGGLYTCQA
CNVLGCARAETLFIIEGAQEKINLEVIILVGTAVIAMFFWLLLVILVRTVKRANEGELKTGYLSIVM
DPDELPLDERCERLPYDASKWEFPRDRLKLGKPLGRGAFGQVIEADAFGIDKTATCKTVAVKMLKEG
ATHSEHRALMSELKILIHIGHHLNVVNLLGACTKPGGPLMVIVEFSKFGNLSTYLRGKRNEFVPYKS
KGARFRQGKDYVGELSVDLKRRLDSITSSQSSASSGFVEEKSLSDVEEEEASEELYKDELTLEHLIC
YSFQVAKGMEFLASRKCIHRDLAARNILLSEKNVVKICDFGLARDIYKDPDYVRKGDARLPLKWMAP
ETIFDRVYTIQSDVWSFGVLLWEIFSLGASPYPGVKIDEEFCRRLKEGTRMRAPDYTTPEMYQTMLD
CWHEDPNQRPSFSELVEHLGNLLQANAQQDGKDYIVLPMSETLSMEEDSGLSLPTSPVSCMEEEEVC
DPKFHYDNTAGISHYLQNSKRKSRPVSVKTFEDIPLEEPEVKVIPDDSQTDSGMVLASEELKTLEDR
NKLSPSFGGMMPSKSRESVASEGSNQTSGYQSGYHSDDTDTTVYSSDEAGLLKMVDAAVHADSGTTL
QLTSCLNGSGPVPAPPPTPGNHERGAA).

Rhesus VEGFR2 used herein comprises an amino acid sequence that can be accessed via the accession number XP_014994176.1 in the NCBI database or an amino acid sequence of SEQ ID NO: 101
(MASKVLLAVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTIKANTTLQITCRGQRDLDWLWPNNQ
SGSEQRVEVTECSDGLFCKTLTIPKVIGNDTGAYKCFYRETDLASVIYVYVQDYRSPFIASVSDQHG
VVYITENKNKTVVIPCLGSISNLNVSLCARYPEKRFVPDGNRISWDSKKGFTIPSYMISYAGMVFCE
AKINDESYQSIMYIVVVVGYRIYDVVLSPSHGVELSVGEKLVLNCTARTELNVGIDENWEYPSSKHQ
HKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKPFVAFGSG
MESLVEATVGERVRIPVKYLGYPPPEIKWYKNGIPLESNHTVKVGHVLTIMEVSERDTGNYTVILTN
PISKEKQSHVVSLVVYVPPQIGEKSLISPVDSYQYGTTQTLTCTVYAIPPPHHIHWYWQLEEECPNE
PSQAVSVTNPYPCEEWRSVEDFQGGNKIEVNKNQFALIEGKNKTVSTLVIQAANVSALYKCEAVNKV
GRGERVISFHVTRGPEITLQPDLQPTEQESVSLWCTADKSTFENLTWYKLGPQPLPVHVGELPTPVC
KNLDTLWKLNATIFSNSINDILIMELKNASLQDQGDYVCVAQDRKTKKRHCVVRQLTVLERVAPMIT
GNLENQTTSIGETIEVSCTASGNPPPQIMWEKDNETLVEDSGIVLKDGNRNLTIRRVRKEDEGLYTC
QACSVLGCAKVEAFFIIEGAQEKTNLEIIILVGTAVIAMFFWLLLVIILRTVKRANGGELKTGYLSI
VMDPDELPLDEHCERLPYDASKWEFPRDRLKLGKPLGRGAFGQVIEADAFGIDKTATCRTVAVKMLK
EGATHSEHRALMSELKILIHIGHHLNVVNLLGACTKPGGPLMVIVEFCKFGNLSTYLRSKRNEFVPY
KTKGARFRQGKDYVGAIPVDLKRRLDSITSSQSSASSGFVEEKSLSDVEEEEAPEDLYKDELTLEHL
ICYSFQVAKGMEFLASRKCIHRDLAARNILLSEKNVVKICDFGLARDIYKDPDYVRKGDARLPLKWM
APETIFDRVYTIQSDVWSFGVLLWEIFSLGASPYPGVKIDEEFCRRLKEGTRMRAPDYTTPEMYQTM
LDCWHGEPSQRPTFSELVEHLGNLLQANAQQDGKDYIVLPISETLSMEEDSGLSLPTSPVSCMEEEE
VCDPKFHYDNTAGISQYLQNSKRKSRPVSVKTFEDIPLEEPEVKVIPDDNQTDSGMVLASEELKTLE
DRTKLAPSFSGMVSSKSRESVASEGSNQTSGYQSGYHSDDTDTTVYSSEEAELLKLIEIGVQTGSTA
QILQPDSGTTLSSPPV)

[0154] A "hVEGFR2-related" disease or condition as used herein refers to any disease or condition caused by, exacerbated by, or otherwise linked to increased or decreased expression or activities of hVEGFR2. In some embodiments, the hVEGFR2 related condition is, for example, cancer, or an angiogenic disease.

[0155] "VEGF-A", used interchangeably with the term "vascular endothelial growth factor A" refers to a highly conserved dimeric glycoprotein or transcript splice variants thereof, such as VEGFA206, VEGFA189, VEGFA165 and VEGFA121 containing 206, 189, 165 and 121 amino acids respectively. VEGF-A is a member of the VEGF family, which also encompasses other members, such as VEGF-B, VEGF-C, and VEGF-D.

[0156] "Cancer" as used herein refers to any medical condition characterized by malignant cell growth or neoplasm, abnormal proliferation, infiltration or metastasis, and includes both solid tumors and non-solid cancers (e.g. hematologic malignancies) such as leukemia. As used herein "solid tumor" refers to a solid mass of neoplastic and/or malignant cells.

[0157] "Angiogenic disease" as used herein refers to any medical condition associated with abnormalities during angiogenesis, a process of growing new blood vessels. Such abnormalities include, without limitation, excessive angiogenesis, insufficient angiogenesis, improper angiogenesis and damaging angiogenesis. Such medical conditions characterized by e.g., excessive or damaging angiogenesis include, without limitation, cancer, diabetic retinopathies, age-related macular degeneration and atherosclerosis, stroke and myocardial infarction, rheumatoid arthritis.

[0158] The term "pharmaceutically acceptable" indicates that the designated carrier, vehicle, diluent, excipient(s), and/or salt is generally chemically and/or physically compatible with the other ingredients comprising the formulation, and physiologically compatible with the recipient thereof.

[0159] Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X." Numeric ranges are inclusive of the numbers defining the range. Generally speaking, the term "about" refers to the indicated value of the variable and to all values of the variable that are within the experimental error of the indicated value (e.g. within the 95% confidence interval for the mean) or within 10 percent of the indicated value, whichever is greater. Where the term "about" is used within the context of a time period (years, months, weeks, days etc.), the term "about" means that period of time plus or minus one amount of the next subordinate time period (e.g. about 1 year means 11-13 months; about 6 months means 6 months plus or minus 1 week; about 1 week means 6-8 days; etc.), or within 10 percent of the indicated value, whichever is greater.

Anti-hVEGFR2 Antibodies

[0160] The present disclosure provides anti-hVEGFR2 antibodies and antigen-binding fragments thereof. The anti-hVEGFR2 antibodies and antigen-binding fragments provided herein are capable of specifically binding to hVEGFR2 or hVEGFR2-expressing cells. "Specifically binding" as used herein means a binding affinity (e.g. K_D) of $\leq 10^{-6}$ M (e.g., $\leq 5\times10^{-7}$ M, $\leq 2\times10^{-7}$ M, $\leq 10^{-7}$ M, $\leq 5\times10^{-8}$ M, $\leq 2\times10^{-7}$ M, $\leq 10^{-8}$ M, 5×10^{-9} M, $\leq 4\times10^{-9}$ M, $\leq 3\times10^{-9}$ M,$\leq 2\times10^{-9}$ M, or $\leq 10^{-9}$ M).

i. Binding Affinity

[0161] Binding affinity of the anti-hVEGFR2 antibodies and antigen-binding fragments provided herein can be represented by K_D value, which represents the ratio of dissociation rate to association rate (k_{off}/k_{on}) when the binding between the antigen and antigen-binding molecule reaches equilibrium. Low-affinity antibodies generally bind antigen slowly and tend to dissociate readily, whereas high-affinity antibodies generally bind antigen faster and tend to remain bound longer. The antigen-binding affinity (e.g. K_D) can be appropriately determined using any suitable methods known in the art, including, for example, Kinetic Exclusion Assay (KinExA), Biacore, Fortebio or flow cytometry.

[0162] In certain embodiments, the "K_D" or "K_D value" according to the present disclosure is in an embodiment measured by Biacore assay, performed with the anti-hVEGFR2 antibody and hVEGFR2 as described by the following assay that measures solution binding affinity of an anti-hVEGFR2 antibody. In general, the Biacore works by equilibrating a constant amount of one binding partner (CBP) with a varying concentration of the other binding partner (Titrant), and then capture a portion of the free CBP by fluorescence labeled secondary antibody in a short contact time which is less than the time needed for dissociation of the pre-formed CBP-Titrant complex. The fluorescence signals generated from the captured CBP are directly proportional to the concentration of free CBP in the equilibrated samples, and are used to generate a binding curve (percent free CBP vs. total Titrant concentration) when measured in a series. More details are available from Schreiber, G., Fersht, A. R. Nature Structural Biology. 1996, 3(5), 427-431. When anti-hVEGFR2 antibody is used as CBP with a constant amount, then hVEGFR2 protein can be used as the Titrant, or vice versa. In certain embodiments, the K_D of the anti-hVEGFR2 antibody or antigen-binding fragments thereof is determined in accordance to the method as described in Example 16 in the present disclosure.

[0163] Other methods suitable for measurement of Kd may also be used under applicable circumstances, for example, radio labelled antigen-binding assay (see, e.g. Chen, et al., (1999) J. Mol Biol 293:865-881).

[0164] In certain embodiments, the binding affinity of the anti-hVEGFR2 antibody is measured by flow cytometry. In general, hVEGFR2-expressing cells (e.g., HUVECs) are incubated with a range of concentrations of an anti-hVEGFR2 antibody, followed by incubation with a fluorescently labelled secondary antibody, and then analyzed for fluorescent signal intensity. In certain embodiments, the binding affinity of the anti-hVEGFR2 antibody or antigen-binding fragments thereof is determined in accordance to the method as described in Example 6 in the present disclosure.

[0165] In certain embodiments, the anti-hVEGFR2 antibodies and the antigen-binding fragments thereof provided herein specifically bind to hVEGFR2 (or a cell expressing hVEGFR2) at a K_D value of no more than 6, 5, 4, 3, 2, 1 or 0.5 nM as measured by Biacore assay. In certain embodiments, the anti-hVEGFR2 antibodies and the antigen-binding fragments thereof provided herein specifically bind to hVEGFR2 (or a cell expressing hVEGFR2) at a K_D value of no more than 0.089 nM (or no more than 0.001 nM) as measured by Biacore assay.

[0166] Alternatively, binding affinity of the anti-hVEGFR2 antibodies and antigen-binding fragments provided herein to hVEGFR2 can also be represented by "half maximal effective concentration" (EC_{50}) value, which refers to the concentration of an antibody where 50% of its maximal effect (e.g., binding) is observed. The EC_{50} value can be measured by methods known in the art, for example, sandwich assay such as ELISA, Western Blot, flow cytometry assay, and other binding assay. In certain embodiments, the anti-hVEGFR2 antibodies and the fragments thereof provided herein specifically bind to recombinant hVEGFR2 at an EC50 value of no more than 80 ng/ml (or no more than 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, 20, 15, 12, or 10, 9 or 8 ng/ml) as measured by ELISA. In certain embodiments, the anti-hVEGFR2 antibodies and the fragments thereof provided herein specifically bind to recombinant hVEGFR2 at an EC50 value of no more than 35, 24, 15, 10, 9, 8, 7 or 6 ng/ml as measured by ELISA. In certain embodiments, the anti-hVEGFR2 antibodies and the fragments thereof provided herein cross-reactive to mouse VEGFR2 having an EC50 value of no more than 35 ng/ml as measured by ELISA. In certain embodiments, the anti-hVEGFR2 antibodies and the fragments thereof provided herein binds to

hVEGFR2 at an EC50 value no more than 60% of that of 1121B, as measured by ELISA. In such embodiment, the anti-hVEGFR2 antibodies and the fragments thereof comprises a HCDR1 comprising the sequence of SEQ ID NO: 31, a HCDR2 comprising the sequence of SEQ ID NO: 32 or SEQ ID NO: 37, a HCDR3 comprising the sequence of SEQ ID NO: 33, a LCDR1 comprising the sequence of SEQ ID NO: 28, a LCDR2 comprising the sequence of SEQ ID NO: 29, and a LCDR3 comprising the sequence of SEQ ID NO: 30. In certain embodiments, the anti-hVEGFR2 antibodies and the fragments thereof provided herein cross-reactive to rhesus VEGFR2 having an EC50 value of no more than 30, 25, 20, 15, 10 or 9 ng/ml as measured by ELISA.

[0167] In certain embodiments, the anti-hVEGFR2 antibodies and the fragments thereof provided herein specifically bind to a cell expressing hVEGFR2 (e.g., HUVEC) at an EC50 value of no more than 160 ng/ml (or no more than 150, 140, 130, 120, 110, 100, 90, 80, 70, 65, 55, 50, 45, 40, 35, 30 or 25 ng/ml) as measured by flow cytometry.

[0168] In certain embodiments, the antibodies and antigen-binding fragments thereof provided herein exhibits competitive VEGFR2 binding property that effectively blocks binding of VEGFR2 to VEGF-A. The blocking effect of the antibodies and antigen-binding fragments thereof provided herein can be measured using ELISA as described in, for example, Example 5 of the present disclosure. In certain embodiments, the blocking effect of the antibodies and antigen-binding fragments thereof provided herein can be expressed in IC50, which indicates the concentration of the antibodies and antigen-binding fragments thereof provided herein at which the binding of VEGFR2 to VEGF-A is decreased by 50% in presence of the antibodies and antigen-binding fragments thereof of the present disclosure. In certain embodiments, the IC50 of the antibodies and antigen-binding fragments thereof provided herein is in a range from 0.001 μg/ml to 2.5 μg/ml, from 0.005 μg/ml to 0.5 μg/ml, from 0.05 μg/ml to 0.4 μg/ml or from 0.1 μg/ml to 0.2 μg/ml. In certain embodiments, the antibodies and antigen-binding fragments thereof provided herein blocks VEGF-A induced proliferation of hVEGFR2-expressing cells at an IC50 value no more than 82% of that of 1121B, as measured by cell viability assay. In such embodiment, the antibodies and antigen-binding fragments thereof provided herein comprises the HCDR1 comprises the sequence of SEQ ID NO: 31, the HCDR2 comprises the sequence of SEQ ID NO: 37, and the HCDR3 comprises the sequence of SEQ ID NO: 33, the LCDR1 comprises the sequence of SEQ ID NO: 28, the LCDR2 comprises the sequence of SEQ ID NO: 29, and the LCDR3 comprises the sequence of SEQ ID NO: 30, preferably expressed in CHO.

Epitope

[0169] In certain embodiments, the anti-hVEGFR2 antibody or an antigen-binding fragment thereof provided herein binds to an epitope comprising at least one or more (e.g. one, two, three or more) of amino acid residues at positions Y137A, R164A, Y165A, V218A, Y221A, R222A, E251A, L252A, N253A, G255A, D257A, K286A, G312A, L313A, M314A, T315A and K316A of hVEGFR2 having the amino acid sequence of SEQ ID NO: 103.

[0170] The term "epitope" as used herein refers to the specific group of atoms or amino acids on an antigen to which an antibody binds. An epitope can include specific amino acids, sugar side chains, phosphoryl or sulfonyl groups that directly contact an antibody. Those skilled in the art will recognize that it is possible to determine, without undue experimentation, if an antibody binds to the same or overlapping or adjacent epitope as the antibody of present disclosure (e.g., hybridoma/chimeric or humanized antibodies 002, 003, 006, 018, 042, 048 and 054 and any of the chimeric and humanized variant thereof provided herein) by ascertaining whether the two competes for binding to a hVEGFR2 antigen polypeptide.

[0171] The term "compete for binding" as used herein with respect to two antigen-binding proteins (e.g. antibodies), means that one antigen-binding protein blocks or reduces binding of the other to the antigen (e.g., human/mouse/rhesus VEGFR2), as determined by a competitive binding assay. Competitive binding assays are well known in the art, include, for example, direct or indirect radioimmunoassay (RIA), direct or indirect enzyme immunoassay (EIA), and sandwich competition assay (see, e.g., Stahli et al., 1983, *Methods in Enzymology* 9:242-253). Typically, such an assay involves the use of purified antigen bound to a solid surface or cells bearing the antigen, an unlabelled test antibody and a labeled reference antibody. Competitive inhibition is measured by determining the amount of label bound to the solid surface or cells in the presence of the test antibody. Usually the test antibody is present in excess. If two antibodies competes for binding to the hVEGFR2, then the two antibodies bind to the same or overlapping epitope, or an adjacent epitope sufficiently proximal to the epitope bound by the other antibody for steric hindrance to occur. Usually, when a competing antibody is present in excess, it will inhibit (e.g., reduce) specific binding of a test antibody to a common antigen by at least 50-55%, 55-60%, 60-65%, 65-70%, 70-75% 75-80%, 80-85%, 85-90% or more.

[0172] In certain embodiments, the epitope or the amino acid residue in the epitope bound by an antibody can be determined by mutating specific residues in the antigen, i.e., hVEGFR2. If an antibody binds to a mutant hVEGFR2 having an amino acid residue mutated, for example to alanine, at significantly reduced level relative to its binding to wild-type hVEGFR2, then this would indicate that the mutated residue is directly involved in the binding of the antibody to hVEGFR2 antigen, or is in close proximity to the antibody when it is bound to the antigen. Such a mutated residue is considered to be within the epitope, and the antibody is considered to specifically bind to an epitope comprising the residue. A significantly reduced level in binding as used herein, means that the binding affinity (e.g. EC50, KD, or binding capacity) between the antibody and the mutant hVEGFR2 is reduced by greater than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more, relative to the binding between the antibody and a wild type hVEGFR2. Such a binding measurement can be conducted using any suitable methods known in the art and disclosed herein, for example, without limitation, KinExA assay, ELISA, Biacore, Fortebio and flow cytometry.

[0173] In certain embodiments, the anti-hVEGFR2 antibody or an antigen-binding fragment thereof provided herein exhibit reduced binding (e.g. at least 30% reduced, 50% reduced) to a mutant hVEGFR2 as measured by ELISA, in which a residue in a wild-type hVEGFR2 is substituted with alanine, and the residue is selected from the group consisting of: Y137A, R164A, Y165A, V218A, Y221A, R222A,

E251A, L252A, N253A, G255A, D257A, K286A, G312A, L313A, M314A, T315A and K316A relative to SEQ ID NO: 103.

[0174] In certain embodiments, the anti-hVEGFR2 antibody or an antigen-binding fragment thereof provided herein binds to an epitope on hVEGFR2, wherein the epitope comprises one or more amino acid residue selected from the group consisting of: Y137, R164, Y165, V218, Y221, R222, E251, L252, N253, G255, D257, K286, G312, L313, M314, T315 and K316 of SEQ ID NO: 103.

[0175] In certain embodiments, the anti-hVEGFR2 antibody or an antigen-binding fragment thereof provided herein binds to an epitope comprising Y165 and/or L313 but not any of R222, G255, D257 and T315 of SEQ ID NO: 103.

[0176] In certain embodiments, the anti-hVEGFR2 antibody or an antigen-binding fragment thereof provided herein binds to an epitope comprising Y137, V218, Y221, R222, E251, L252, N253, G255, D257, K286, L313, M314, T315, K316 or any combination thereof of SEQ ID NO: 103.

[0177] In certain embodiments, the anti-hVEGFR2 antibody or an antigen-binding fragment thereof provided herein binds to an epitope comprising R164, Y165, D257 or any combination thereof of SEQ ID NO: 103.

[0178] In certain embodiments, the anti-hVEGFR2 antibody or an antigen-binding fragment thereof provided herein binds to an epitope comprising Y165, Y221, R222, E251, G255, D257, G312, L313, M314, T315, K316 or any combination thereof of SEQ ID NO: 103.

[0179] In certain embodiments, the anti-hVEGFR2 antibody or an antigen-binding fragment thereof provided herein binds to an epitope comprising Y165 except R222, G255, D257, L313 and T315 of SEQ ID NO: 103.

[0180] In certain embodiments, the anti-hVEGFR2 antibody or an antigen-binding fragment thereof provided herein binds to an epitope comprising Y165, Y221, R222, E251, N253, G255, D257, G312, L313, M314, T315, K316 or any combination thereof of SEQ ID NO: 103.

[0181] In certain embodiments, the anti-hVEGFR2 antibody or an antigen-binding fragment thereof provided herein binds to an epitope comprising R164, Y165, D257 or any combination thereof of SEQ ID NO: 103.

[0182] In certain embodiments, the anti-hVEGFR2 antibody or an antigen-binding fragment thereof provided herein binds to an epitope comprising Y165, Y221, R222, E251, N253, G255, D257, G312, L313, M314, T315, K316 or any combination thereof of SEQ ID NO: 103.

Antibody Sequences

[0183] In another aspect, the present disclosure provides an anti-hVEGFR2 antibody or an antigen-binding fragment thereof, comprising heavy chain HCDR1, HCDR2 and HCDR3 and/or light chain LCDR1, LCDR2 and LCDR3 sequences, wherein

[0184] the HCDR1 sequence comprises SSWMN (SEQ ID NO: 1), DYYMS (SEQ ID NO: 19), X1YGMS (SEQ ID NO: 41), X4YWIM (SEQ ID NO: 44), or a homologue sequence of at least 80% sequence identity thereof,

[0185] the HCDR2 sequence comprises RIFPGDGDTYYNGKFQV (SEQ ID NO: 2), FIRNKANGYTTEYSASVKG (SEQ ID NO: 20), SISX$_2$GGSYTYYADSVX$_{19}$G (SEQ ID NO: 42), DIYPGX$_5$GSTNYNEKFKS (SEQ ID NO: 45) or a homologue sequence of at least 80% sequence identity thereof,

[0186] the HCDR3 sequence comprises FLDTSGRYVDY (SEQ ID NO: 3), FDYYGSTYCFDY (SEQ ID NO: 21), EX$_3$DGNYDY (SEQ ID NO: 43), DSNPDY (SEQ ID NO: 46), or a homologue sequence of at least 80% sequence identity thereof;

[0187] the LCDR1 sequence comprises KASQDVNTAVA (SEQ ID NO: 4), RASQSVSTSSSSFMH (SEQ ID NO: 22), RSSKSLLYKDGKTYLN (SEQ ID NO: 28), RASESVX$_6$NSGISFMX$_7$ (SEQ ID NO: 47) or a homologue sequence of at least 80% sequence identity thereof,

[0188] the LCDR2 sequence comprises SASYRYI (SEQ ID NO: 5), YASNLES (SEQ ID NO: 23), LMSTRAS (SEQ ID NO: 29), AASX$_8$QX$_9$S (SEQ ID NO: 48) or a homologue sequence of at least 80% sequence identity thereof;

[0189] the LCDR3 sequence comprises QQHYRAPLT (SEQ ID NO: 6), QHTWEIPLT (SEQ ID NO: 24), QQLVEYPFT (SEQ ID NO: 30), QQSKEVPYT (SEQ ID NO: 49) or a homologue sequence of at least 80% sequence identity thereof;

[0190] wherein X1 is I or M, X$_2$ is V or I, X$_3$ is L or M, X$_4$ is T or S, X$_5$ is T or S, X$_6$ is D or E, X$_7$ is T or H, X$_8$ is T or Y, X$_9$ is G or R, and X$_{19}$ is E or K.

[0191] In one aspect, the present disclosure provides an anti-hVEGFR2 antibody or an antigen-binding fragment thereof provided herein, wherein the HCDR1 comprises an amino acid sequence of SEQ ID NO: 41, the HCDR2 comprises an amino acid sequence of SEQ ID NO: 42, the HCDR3 comprises an amino acid sequence of SEQ ID NO: 43, the LCDR1 comprises a sequence of SEQ ID NO: 28, the LCDR2 comprises a sequence of SEQ ID NO: 29, and the LCDR3 comprises a sequence of SEQ ID NO: 30.

[0192] In one aspect, the present disclosure provides an anti-hVEGFR2 antibody or an antigen-binding fragment thereof provided herein, wherein

[0193] a) the HCDR1 comprises the sequence of SEQ ID NO: 25, a HCDR2 comprises the sequence of SEQ ID NO: 26, the HCDR3 comprises the sequence of SEQ ID NO: 27; the LCDR1 comprises the sequence of SEQ ID NO: 28, the LCDR2 comprises the sequence of SEQ ID NO: 29, and the LCDR3 comprises the sequence of SEQ ID NO: 30; or

[0194] b) the HCDR1 comprises the sequence of SEQ ID NO: 31, the HCDR2 comprises the sequence of SEQ ID NO: 32 or SEQ ID NO: 37, and the HCDR3 comprises the sequence of SEQ ID NO: 33, the LCDR1 comprises the sequence of SEQ ID NO: 28, the LCDR2 comprises the sequence of SEQ ID NO: 29, and the LCDR3 comprises the sequence of SEQ ID NO: 30; or

[0195] c) the HCDR1 comprises the sequence of SEQ ID NO: 34, the HCDR2 comprises the sequence of SEQ ID NO: 35 or SEQ ID NO: 37, and the HCDR3 comprises the sequence of SEQ ID NO: 36, the LCDR1 comprises the sequence of SEQ ID NO: 28, the LCDR2 comprises the sequence of SEQ ID NO: 29, and the LCDR3 comprises the sequence of SEQ ID NO: 30.

[0196] In one aspect, the present disclosure provides an anti-hVEGFR2 antibody or an antigen-binding fragment thereof provided herein, wherein the HCDR1 comprises an amino acid sequence of SEQ ID NO: 44, the HCDR2

comprises an amino acid sequence of SEQ ID NO: 45, the HCDR3 comprises an amino acid sequence of SEQ ID NO: 46, the LCDR1 comprises a sequence of SEQ ID NO: 47, the LCDR2 comprises a sequence of SEQ ID NO: 48, and the LCDR3 comprises a sequence of SEQ ID NO: 49.

[0197] In one aspect, the present disclosure provides an anti-hVEGFR2 antibody or an antigen-binding fragment thereof provided herein, wherein

[0198] a) the HCDR1 comprises the sequence of SEQ ID NO: 7, a HCDR2 comprises the sequence of SEQ ID NO: 8, the HCDR3 comprises the sequence of SEQ ID NO: 9; the LCDR1 comprises the sequence of SEQ ID NO: 10, the LCDR2 comprises the sequence of SEQ ID NO: 11, and the LCDR3 comprises the sequence of SEQ ID NO: 12; or

[0199] b) the HCDR1 comprises the sequence of SEQ ID NO: 13, the HCDR2 comprises the sequence of SEQ ID NO: 14, and the HCDR3 comprises the sequence of SEQ ID NO: 15, the LCDR1 comprises the sequence of SEQ ID NO: 16, the LCDR2 comprises the sequence of SEQ ID NO: 17, and the LCDR3 comprises the sequence of SEQ ID NO: 18.

[0200] In one aspect, the present disclosure provides an anti-hVEGFR2 antibody or an antigen-binding fragment thereof provided herein, wherein

[0201] a) the HCDR1 comprises the sequence of SEQ ID NO: 1, the HCDR2 comprises the sequence of SEQ ID NO: 2, and the HCDR3 comprises the sequence of SEQ ID NO: 3, the LCDR1 comprises the sequence of SEQ ID NO: 4, the LCDR2 comprises the sequence of SEQ ID NO: 5, and the LCDR3 comprises the sequence of SEQ ID NO: 6; or

[0202] b) the HCDR1 comprises the sequence of SEQ ID NO: 19, the HCDR2 comprises the sequence of SEQ ID NO: 20, and the HCDR3 comprises the sequence of SEQ ID NO: 21, the LCDR1 comprises the sequence of SEQ ID NO: 22, the LCDR2 comprises the sequence of SEQ ID NO: 23, and the LCDR3 comprises the sequence of SEQ ID NO: 24.

[0203] In certain embodiments, the antibodies provided herein comprise one or more (e.g. 1, 2, 3, 4, 5, or 6) CDR sequences of a hVEGFR2 antibodies 002, 003, 006, 018, 042, 048 and 054.

[0204] "002" or "2" as used herein with respect to an antibody refers to a mouse antibody having a heavy chain variable region of SEQ ID NO: 50, and a light chain variable region of SEQ ID NO: 51.

[0205] "003" or "3" as used herein with respect to an antibody refers to a mouse antibody having a heavy chain variable region of SEQ ID NO: 52, and a light chain variable region of SEQ ID NO: 53.

[0206] "006" or "6" as used herein with respect to an antibody refers to a mouse antibody having a heavy chain variable region of SEQ ID NO: 54, and a light chain variable region of SEQ ID NO: 55.

[0207] "018" or "18" as used herein with respect to an antibody refers to a mouse antibody having a heavy chain variable region of SEQ ID NO: 56, and a light chain variable region of SEQ ID NO: 57.

[0208] "042" or "42" as used herein with respect to an antibody refers to a mouse antibody having a heavy chain variable region of SEQ ID NO: 58, and a light chain variable region of SEQ ID NO: 59.

[0209] "048" or "48" as used herein with respect to an antibody refers to a mouse antibody having a heavy chain variable region of SEQ ID NO: 60, and a light chain variable region of SEQ ID NO: 61.

[0210] "054" or "45" as used herein with respect to an antibody refers to a mouse antibody having a heavy chain variable region of SEQ ID NO: 62, and a light chain variable region of SEQ ID NO: 63.

[0211] Table 1 shows the CDR sequences of these hVEGFR2 antibodies. The heavy chain and light chain variable region sequences are also provided below in Table 2.

TABLE 1

Sequences of hVEGFR2 antibodies' CDR region

	Region	CDR1	CDR2	CDR3
002	HCDR	SEQ ID NO: 1 SSWMN	SEQ ID NO: 2 RIFPGDGDTYYNGK FQV	SEQ ID NO: 3 FLDTSGRYVDY
	LCDR	SEQ ID NO: 4 KASQDVNTAVA	SEQ ID NO: 5 SASYRYI	SEQ ID NO: 6 QQHYRAPLT
003	HCDR	SEQ ID NO: 7 TYWIM	SEQ ID NO: 8 DIYPGTGSTNYNEKF KS	SEQ ID NO: 9 DSNPDY
	LCDR	SEQ ID NO: 10 RASESVDNSGISFMT	SEQ ID NO: 11 AASTQGS	SEQ ID NO: 12 QQSKEVPYT
006	HCDR	SEQ ID NO: 13 SYWIM	SEQ ID NO: 14 DIYPGSGSTNYNEKF KS	SEQ ID NO: 15 DSNPDY
	LCDR	SEQ ID NO: 16 RASESVENSGISFMH	SEQ ID NO: 17 AASYQRS	SEQ ID NO: 18 QQSKEVPYT
018	HCDR	SEQ ID NO: 19 DYYMS	SEQ ID NO: 20 FIRNKANGYTTEYSA SVKG	SEQ ID NO: 21 FDYYGSTYCFDY
	LCDR	SEQ ID NO: 22 RASQSVSTSSSSFMH	SEQ ID NO: 23 YASNLES	SEQ ID NO: 24 QHTWEIPLT

TABLE 1-continued

Sequences of hVEGFR2 antibodies' CDR region

	Region	CDR1	CDR2	CDR3
042	HCDR	SEQ ID NO: 25 IYGMS	SEQ ID NO: 26 SISVGGSYTYYADSV EG	SEQ ID NO: 27 ELDGNYDY
	LCDR	SEQ ID NO: 28 RSSKSLLYKDGKTYL N	SEQ ID NO: 29 LMSTRAS	SEQ ID NO: 30 QQLVEYPFT
048	HCDR	SEQ ID NO: 31 MYGMS	SEQ ID NO: 32 SISIGGSYTYYADSVE G SEQ ID NO: 37 SISIGGSYTYYADSV<u>K</u> G	SEQ ID NO: 33 EMDGNYDY
	LCDR	SEQ ID NO: 28 RSSKSLLYKDGKTYL N	SEQ ID NO: 29 LMSTRAS	SEQ ID NO: 30 QQLVEYPFT
054	HCDR	SEQ ID NO: 34 MYGMS	SEQ ID NO: 35 SISIGGSYTYYADSVE G SEQ ID NO: 37 SISIGGSYTYYADSV<u>K</u> G	SEQ ID NO: 36 ELDGNYDY
	LCDR	SEQ ID NO: 28 RSSKSLLYKDGKTY LN	SEQ ID NO: 29 LMSTRAS	SEQ ID NO: 30 QQLVEYPFT
042 & 048 & 054	HCDR	SEQ ID NO: 41 X_1YGMS	SEQ ID NO: 42 SISX_2GGSYTYYADSV X_{19}G	SEQ ID NO: 43 EX_3DGNYDY
	LCDR	SEQ ID NO: 28 RSSKSLLYKDGKTYL N	SEQ ID NO: 29 LMSTRAS	SEQ ID NO: 30 QQLVEYPFT
003 & 006	HCDR	SEQ ID NO: 44 X_4YWIM	SEQ ID NO: 45 DIYPGX_5GSTNYNEK FKS	SEQ ID NO: 46 DSNPDY
	LCDR	SEQ ID NO: 47 RASESVX_6NSGISFMX_7	SEQ ID NO: 48 AASX_8QX_9S	SEQ ID NO: 49 QQSKEVPYT

wherein X_1 is I or M, X_2 is V or I, X_3 is L or M, X_4 is T or S, X_5 is T or S, X_6 is D or E, X_7 is T or H, X_8 is T or Y, X_9 is G or R, and X_{19} is E or K.

TABLE 2

Sequences of mouse/chimeric antibody VH/VL

	VH	VL
002	SEQ ID NO: 50 QVQLQQSGPELVKPGASVKISCKASGYAFS**SS** **WMN**WVKQRPGEGLEWIG**RIFPGDGDTYYN** **GKFQV**KATLTADKSSSTAYMQLSSLTSEDSAV YFCAI**FLDTSGRYVDY**WGQGTTLTISS	SEQ ID NO: 51 DIVMTQSHKFMSTSVGDRVSITC**KASQDVNT** **AVA**WYQQKPGQSPKLLIY**SASYRYI**GVPDRFT GSGSGTDFTFTISSVQSEDLTVYYC**QQHYRAP** **LT**FGSGTKLELK
003	SEQ ID NO: 52 QVQLQQPGAELVKPGASVKMSCKASGYTFN **TYWIM**WVKQRPGQGLEWIG**DIYPGTGSTNY** **NEKFKS**KVTLTADTSSTTAYMQVSSLTSEDSA VYYCGR**DSNPDY**WGQGTTLTVSS	SEQ ID NO: 53 DIVLTQSPASLAVSLGQRATISC**RASESVDNSG** **ISFMT**WFQQKPGQPPKLLIY**AASTQGS**GVPA RFSGSGSGTDFSLNIHPVEEDDTAMYFC**QQSK** **EVPYT**FGGGTKLEIK
006	SEQ ID NO: 54 QAQLQQPGAELVKPGTSVKMSCKASGYTEN**S** **YWIM**WVKQSPGQGLEWIG**DIYPGSGSTNYN** **EKFKS**KVTLTVDTSSSTAYMQVSSLTSEDSAV YYCAR**DSNPDY**WGQGTTLTVSS	SEQ ID NO: 55 DIVLTQSPASLAVSLGQRATISC**RASESVENSG** **ISFMH**WFQQKPGQPPKLLIY**AASYQRS**GVPA RFSGSGSGTDFSLNIHPVEEDDIAMYFC**QQSK** **EVPYT**FGGGTKLEIK

TABLE 2-continued

Sequences of mouse/chimeric antibody VH/VL

	VH	VL
018	SEQ ID NO: 56 EVKLVESGGGLVQPGGSLSLSCAVSGFTFT**DY** **YMS**WVRQPPGKALEWLG**FIRNKANGYTTE** **YSASVKG**RFTISRDNSQSILYLQMNALRAEDS ATYYCAR**FDYYGSTYCFDY**WGQGTTLTVSS	SEQ ID NO: 57 DIVLTQSPASLAVSLGQRATISC**RASQSVSTSS** **SSFMH**WYQQKPGQPPKLLIK**YASNLES**GVPA RFSGSGSGTDFTLNIHPVEEEDTATYYC**QHT** **WEIPLT**FGAGTKLELK
042	SEQ ID NO: 58 EVQLVESGGDLVKPGGSLKLSCTASGFSFS**IY** **GMS**WVRQTPDKRLEWVA**SISVGGSYTYYAD** **SVEG**RFTISRENAKNTLYLQMNSLKSEDTALY YCAR**ELDGNYDY**WGQGTSLTVSS	SEQ ID NO: 59 DIVITQNELSNPVTFGESVSISC**RSSKSLLYKD** **GKTYLN**WFLQRPGQSPQLLIY**LMSTRAS**GVS DRFSGSGSGTDFTLEISRVKAEDVGVYYC**QQ** **LVEYPFT**FGSGTKLEIK
048	SEQ ID NO: 60 EVQLVESGGDLVKPGGSLKLSCAASGFTF**SM** **YGMS**WVRQTPDKRLEWVA**SISIGGSYTYYA** **DSVEG**RFTISRENAKNTLFLQMNSLKSEDTAL YYCAR**EMDGNYDY**WGHGTTLTVSS	SEQ ID NO: 61 DVMITQDELSNPVTFGESVSISC**RSSKSLLYK** **DGKTYLN**WFLQRPGQSPQLLIY**LMSTRAS**G VSDRFSGSGSGTDFTLEISRVKAEDVGIYYC**Q** **QLVEYPFT**FGSGTKLEIK
054	SEQ ID NO: 62 EVQLVESGGDLVKPGGSLKLSCAASGFTFS**M** **YGMS**WVRQTPDKRLEWVA**SISIGGSYTYYA** **DSVEG**RFIISRENAKNTLFLQMNSLKSEDTAL YYCAR**ELDGNYDY**WGQGTTLTVSS	SEQ ID NO: 63 HIMITQDELSNPVTFGESVSISC**RSSKSLLYKD** **GKTYLN**WFLQRPGQSPQLLIY**LMSTRAS**GVS DRFSGSGSGTDFTLEISRVKAEDVGIYYC**QQL** **VEYPFT**FGSGTKLEIK

wherein the CDR regions are bolded.

[0212] The anti-hVEGFR2 antibodies or antigen-binding fragments thereof provided herein can be a monoclonal antibody, polyclonal antibody, humanized antibody, chimeric antibody, recombinant antibody, bispecific antibody, labeled antibody, bivalent antibody, or anti-idiotypic antibody. A recombinant antibody is an antibody prepared in vitro using recombinant methods rather than in animals.

[0213] CDRs are known to be responsible for antigen binding, however, it has been found that not all of the 6 CDRs are necessarily indispensable or unchangeable. In other words, it is possible to replace or change or modify 1, 2, or 3 CDRs in anti-hVEGFR2 antibodies provided herein, yet substantially retain the specific binding affinity to hVEGFR2.

[0214] In certain embodiments, the anti-hVEGFR2 antibodies and the antigen-binding fragments provided herein comprise a heavy chain CDR3 sequence of one of the anti-hVEGFR2 antibodies 002, 003, 006, 018, 042, 048 and 054. Heavy chain CDR3 regions are located at the center of the antigen-binding site, and therefore are believed to make the most contact with antigen and provide the most free energy to the affinity of antibody to antigen. It is also believed that the heavy chain CDR3 is by far the most diverse CDR of the antigen-binding site in terms of length, amino acid composition and conformation by multiple diversification mechanisms (Tonegawa S. Nature. 302:575-81). The diversity in the heavy chain CDR3 is sufficient to produce most antibody specificities (Xu J L, Davis M M. Immunity. 13:37-45) as well as desirable antigen-binding affinity (Schier R, etc. J Mol Biol. 263:551-67).

[0215] In some embodiments, the anti-hVEGFR2 antibodies and the antigen-binding fragments provided herein comprise all or a portion of the heavy chain variable domain and/or all or a portion of the light chain variable domain. In one embodiment, the anti-hVEGFR2 antibodies and the antigen-binding fragments provided herein is a single domain antibody which consists of all or a portion of the heavy chain variable domain provided herein. More information of such a single domain antibody is available in the art (see, e.g., U.S. Pat. No. 6,248,516).

[0216] In certain embodiments, the antibodies and antigen-binding fragments thereof provided herein comprise suitable framework region (FR) sequences, as long as the antibodies and antigen-binding fragments thereof can specifically bind to hVEGFR2. The CDR sequences provided in Table 1 are obtained from mouse antibodies, but they can be grafted to any suitable FR sequences of any suitable species such as mouse, human, rat, rabbit, among others, using suitable methods known in the art such as recombinant techniques.

[0217] In certain embodiments, the antibodies and antigen-binding fragments thereof provided herein are humanized. A humanized antibody or antigen-binding fragment is desirable in its reduced immunogenicity in human. A humanized antibody is chimeric in its variable regions, as non-human CDR sequences are grafted to human or substantially human FR sequences. Humanization of an antibody or antigen-binding fragment can be essentially performed by substituting the non-human (such as murine) CDR genes for the corresponding human CDR genes in a human immunoglobulin gene (see, for example, Jones et al. (1986) Nature 321:522-525; Riechmann et al. (1988) Nature 332:323-327; Verhoeyen et al. (1988) Science 239:1534-1536).

[0218] Suitable human heavy chain and light chain variable domains can be selected to achieve this purpose using methods known in the art. In an illustrative example, "best-fit" approach can be used, where a non-human (e.g., rodent) antibody variable domain sequence is screened or BLASTed against a database of known human variable domain germline sequences, and the human sequence closest to the non-human query sequence is identified and used as the human scaffold for grafting the non-human CDR sequences (see, for example, Sims et al, (1993) J. Immunol. 151:2296; Chothia et al. (1987) J. Mot. Biol. 196:901). Alternatively, a

framework derived from the consensus sequence of all human antibodies may be used for the grafting of the non-human CDRs (see, for example, Carter et al. (1992) Proc. Natl. Acad. Sci. USA, 89:4285; Presta et al. (1993) J. Immunol., 151:2623).

[0219] In certain embodiments, the humanized antibodies or antigen-binding fragments provided herein are composed of substantially all human sequences except for the CDR sequences which are non-human. In some embodiments, the variable region FRs, and constant regions if present, are entirely or substantially from human immunoglobulin sequences. The human FR sequences and human constant region sequences may be derived different human immunoglobulin genes, for example, FR sequences derived from one human antibody and constant region from another human antibody. In some embodiments, the humanized antibody or antigen-binding fragment comprise human heavy/light chain FR1-4.

[0220] In some embodiments, the FR regions derived from human may comprise the same amino acid sequence as the human immunoglobulin from which it is derived. In some embodiments, one or more amino acid residues of the human FR are substituted with the corresponding residues from the parent non-human antibody. This may be desirable in certain embodiments to make the humanized antibody or its fragment closely approximate the non-human parent antibody structure to reduce or avoid immunogenicity and/or improve or retain the binding activity or binding affinity.

[0221] In certain embodiments, the humanized antibody or antigen-binding fragment provided herein comprises no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residue substitutions in each of the human FR sequences, or no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residue substitutions in all the FRs of a heavy or a light chain variable domain. In some embodiments, such change in amino acid residue could be present in heavy chain FR regions only, in light chain FR regions only, or in both chains. In certain embodiments, the one or more amino acid residues are mutated, for example, back-mutated to the corresponding residue found in the non-human parent antibody (e.g. in the mouse framework region) from which the CDR sequences are derived. Suitable positions for mutations can be selected by a skilled person following principles known in the art. For example, a position for mutation can be selected where: 1) the residue in the framework of the human germline sequence is rare (e.g. in less than 20% or less than 10% in human variable region sequence); 2) the position is immediately adjacent to one or more of the 3 CDR's in the primary sequence of the human germline chain, as it is likely to interact with residues in the CDRs; or 3) the position is close to CDRs in a 3-dimensional model, and therefore can have a good probability of interacting with amino acids in the CDR. The residue at the selected position can be mutated back to the corresponding residue in the parent antibody, or to a residue which is neither the corresponding residue in human germline sequence nor in parent antibody, but to a residue typical of human sequences, i.e. that occurs more frequently at that position in the known human sequences belonging to the same subgroup as the human germline sequence (see U.S. Pat. No. 5,693,762).

[0222] In certain embodiments, the humanized light and heavy chains of the present disclosure are substantially non-immunogenic in humans and retain substantially the same affinity as or even higher affinity than the parent antibody to hVEGFR2.

[0223] In certain embodiments, the humanized antibodies and antigen-binding fragment thereof provided herein comprise one or more light chain FR sequences of human germline framework sequence VK/2D-40, and/or one or more heavy chain FR sequences of human germline framework sequence V_H/3-21, with or without back mutations. Back mutations can be introduced into the human germline framework sequence, if needed. In certain embodiments, the humanized antibody 054 may contain one or more back mutations selected from the group consisting of: R19K, A40T, G44R, S49A, S78T, and R87K relative to the framework sequences in SEQ ID NO: 92 (EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYS-MNWVRQAPGKGLEWVSSISSSSSYIYYADSVKGR FTISRDNAKNSLYLQMNSLPAEDTAVYY-CARELDGNYDYWGQGTTLTVSS) of the heavy chain framework sequence V_H/3-21. The humanized antibody 048 may contain one or more back mutations selected from the group consisting of: G44R, S49A and S78T relative to the framework sequences in SEQ ID NO: 92 (EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYS-MNWVRQAPGKGLEWVSSISSSSSYIYYADSVKGR FTISRDNAKNSLYLQMNSLPAEDTAVYY-CARELDGNYDYWGQGTTLTVSS) of the heavy chain framework sequence V_H/3-21. In certain embodiments, the humanized antibody 054/048 may contain one or more back mutations selected from the group consisting of: M4T, T7D, P8E, P15F, P18S, A19V, Y42F, K45R, P65S and V91I relative to the framework sequences in SEQ ID NO: 95 (DIVMTQTPLSLPVTPGEPAS ISCRSSQSLLDSDDGN-TYLDWYLQKPGQSPQLLIYTLSYPASGVPDR FSGSGSGTDFTLKISRVEAEDVGVYYCMQRIEFP) of the light chain framework sequence VK/2D-40.

[0224] In certain embodiments, the anti-hVEGFR2 antibody or an antigen-binding fragment thereof provided herein, comprises a heavy chain variable region comprising the sequence selected from the group consisting of SEQ ID NO: 93, SEQ ID NO: 94 and SEQ ID NO: 98, and a homologous sequence thereof having at least 80% (e.g. at least 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity yet retaining specific binding affinity to hVEGFR2, in particular human hVEGFR2.

[0225] In certain embodiments, the anti-hVEGFR2 antibody or an antigen-binding fragment thereof provided herein, antibody or an antigen-binding fragment thereof comprises a light chain variable region comprising the sequence selected from the group consisting of SEQ ID NO: 96 and SEQ ID NO: 97, and a homologous sequence thereof having at least 80% (e.g. at least 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity yet retaining specific binding affinity to hVEGFR2, in particular human hVEGFR2.

[0226] In certain embodiments, the anti-hVEGFR2 antibody or an antigen-binding fragment thereof provided herein, comprising: a heavy chain variable region comprising the sequence selected from the group consisting of SEQ ID NO: 93, SEQ ID NO: 94 and SEQ ID NO: 98, and a light chain variable region comprising the sequence of SEQ ID NO: 96 or SEQ ID NO: 97.

[0227] In certain embodiments, the anti-hVEGFR2 antibody or an antigen-binding fragment thereof provided herein

further comprises one or more of heavy chain HFR1, HFR2, HFR3 and HFR4, and/or one or more of light chain LFR1, LFR2, LFR3 and LFR4, wherein:

[0228] the HFR1 comprises EVQLVESGG-GLVKPGGSLXioLSCAASGFTFS (SEQ ID NO: 84) or a homologous sequence of at least 80% (or at least 85%, 90%, 95%) sequence identity thereof,

[0229] the HFR2 comprises WVRQX_{11}PGKRLEWVA (SEQ ID NO: 85) or a homologous sequence of at least 80% (or at least 90%) sequence identity thereof,

[0230] the HFR3 sequence comprises RFTISRDNAKNTLYLQMNSLX_{12}AEDTAVYYCAR (SEQ ID NO: 86) or a homologous sequence of at least 80% (or at least 85%, 90%, 95%) sequence identity thereof,

[0231] the HFR4 comprises WGX_{13}GTTLTVSS (SEQ ID NO: 87) or a homologous sequence of at least 80% sequence identity thereof,

[0232] the LFR1 comprises DIVITQX_{14}X15LSLPVTX_{16}GESVSISC (SEQ ID NO: 88) or a homologous sequence of at least 80% (or at least 85%, 90%, 95%) sequence identity thereof,

[0233] the LFR2 comprises WFLQRPGQSPQLLIY (SEQ ID NO: 89) or a homologous sequence of at least 80% (or at least 85%, 90%) sequence identity thereof,

[0234] the LFR3 comprises GVX_{17}DRFSGSGSGTDFTLKISRVEAEDVGX_{18} YYC (SEQ ID NO: 90) or a homologous sequence of at least 80% (or at least 85%, 90%, 95%) sequence identity thereof, and

[0235] the LFR4 comprises FGSGTKLEK (SEQ ID NO: 91) or a homologous sequence of at least 8000 (or at least 900LG) sequence identity thereof,

[0236] wherein X_{10} is R or K, X_{11} is A or T, X_{12} is R or K, X_{13} is Q or H, X_{14} is D or T, X_{15} is E or P, X_{16} is For P, X_{17} is S or P, X_{18} is V or I.

[0237] In certain embodiments, the HIFR1 comprises a sequence selected from the group consisting of SEQ ID NOs: 64, 68 and 72, the HFR2 comprises a sequence selected from the group consisting of SEQ ID NOs: 65, 69 and 73, the HFR3 comprises the sequence selected from the group consisting of SEQ ID NOs: 66, 70 and 74, the HFR4 comprises a sequence selected from the group consisting of SEQ ID NOs: 67, 71 and 75, the LFR1 comprises the sequence from the group consisting of SEQ ID NOs: 76 and 80, the LFR2 comprises a sequence selected from the group consisting of SEQ ID NO: 77 and 81, the LFR3 comprises a sequence selected from the group consisting of SEQ ID NOs: 78 and 82, and the LFR4 comprises a sequence selected from the group consisting of SEQ ID NOs: 79 and 83.

TABLE 3

Framework (FR) sequences of humanized hVEGFR2 antibodies 054 and 048

Antibody chain	FR1	FR2	FR3	FR4
Mab54-Hzd-HC-V1	SEQ ID NO: 64 EVQLVESGGGL VKPGGSLKLSC AASGFTFS	SEQ ID NO: 65 WVRQTPGKRLE WVA	SEQ ID NO: 66 RFTISRDNAKNT LYLQMNSLKAE DTAVYYCAR	SEQ ID NO: 67 WGQGTTLTVSS
Mab54-HC-V2	SEQ ID NO: 68 EVQLVESGGGL VKPGGSLRLSCA ASGFTFS	SEQ ID NO: 69 WVRQAPGKRLE WVA	SEQ ID NO: 70 RFTISRDNAKNT LYLQMNSLRAE DTAVYYCAR	SEQ ID NO: 71 WGQGTTLTVSS
Mab48-Hzd-HC-V3	SEQ ID NO:72 EVQLVESGGGL VKPGGSLRLSCA ASGFTFS	SEQ ID NO: 73 WVRQAPGKRLE WVA	SEQ ID NO: 74 RFTISRDNAKNT LYLQMNSLRAE DTAVYYCAR	SEQ ID NO: 75 WGHGTTLTVSS
Mab54-Hzd-LC-V1	SEQ ID NO: 76 DIVITQDELSLPV TPGESVSISC	SEQ ID NO: 77 WFLQRPGQSPQ LLIY	SEQ ID NO: 78 GVSDRFSGSGSG TDFTLKISRVEA EDVGIYYC	SEQ ID NO: 79 FGSGTKLEIK
Mab54-Hzd-LC-V2	SEQ ID NO: 80 DIVITQTPLSLPV TPGESVSISC	SEQ ID NO: 81 WFLQRPGQSPQ LLIY	SEQ ID NO: 82 GVPDRFSGSGSG TDFTLKISRVEA EDVGVYYC	SEQ ID NO: 83 FGSGTKLEIK

[0238] Tables 4 and 5 illustrate sequences of the variable regions of humanized 054 and 048 antibodies.

TABLE 4

Sequences of humanized 054

Antibody chain	Sequences
Mab54-germline	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISS SSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARELDGNYDY WGQGTTLTVSS (SEQ ID NO: 92)

TABLE 4-continued

Sequences of humanized 054

Antibody chain	Sequences
Mab54-Hzd-HC-V1	EVQLVESGGGLVKPGGSLKLSCAASGFTFSMYGMSWVRQTPGKRLEWVASISI GGSYTYYADSVKGRFTISRDNAKNTLYLQMNSLKAEDTAVYYCARELDGNYD YWGQGTTLTVSS (SEQ ID NO: 93)
Mab54-HC-V2	EVQLVESGGGLVKPGGSLRLSCAASGFTFSMYGMSWVRQAPGKRLEWVASISI GGSYTYYADSVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCARELDGNYD YWGQGTTLTVSS (SEQ ID NO: 94)
Mab54 LC germline	DIVMTQTPLSLPVTPGEPASISCRSSQSLLDSDDGNTYLDWYLQKPGQSPQLLIY TLSYRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQRIEFP (SEQ ID NO: 95)
Mab54-Hzd-LC-V1	DIVITQDELSLPVTFGESVSISCRSSKSLLYKDGKTYLNWFLQRPGQSPQLLIYL MSTRASGVSDRFSGSGSGTDFTLKISRVEAEDVGIYYCQQLVEYPFTFGSGTKL EIK (SEQ ID NO: 96)
Mab54-Hzd-LC-V2	DIVITQTPLSLPVTPGESVSISCRSSKSLLYKDGKTYLNWFLQRPGQSPQLLIYLM STRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCQQLVEYPFTFGSGTKLEI K (SEQ ID NO: 97)

TABLE 5

Sequences of humanized 048

Antibody chain	Sequences
Mab48-Hzd-HC-V3	EVQLVESGGGLVKPGGSLRLSCAASGFTFSMYGMSWVRQAP GKRLEWVASISIGGSYTYYADSVKGRFTISRDNAKNTLYLQM NSLRAEDTAVYYCAREMDGNYDYWGHGTTLTVSS (SEQ ID NO: 98)

[0239] The light chain variable regions of the humanized 048 are the same of those of the humanized 054.

[0240] The humanized anti-hVEGFR2 antibodies provided herein retained the specific binding affinity to hVEGFR2-expressing cell, and are at least comparable to, or even better than, the parent antibodies in that aspect. The humanized antibodies provided herein can also retain their functional interaction with VEGFR2-expressing cells, such as HUVEC cells, in that all antibodies can inhibit VEGF-A induced VEGFR2 phosphorylation, HUVEC proliferation and tube formation. In certain embodiments, the anti-hVEGFR2 antibodies and the fragments thereof provided herein further comprise an immunoglobulin constant region, optionally a constant region of human Ig, or optionally a constant region of human IgG. In some embodiments, an immunoglobulin constant region comprises a heavy chain and/or a light chain constant region. The heavy chain constant region comprises CH1, hinge, and/or CH2-CH3 regions. In certain embodiments, the heavy chain constant region comprises an Fc region. In certain embodiments, the light chain constant region comprises Cκ or Cλ.

[0241] In certain embodiments, the anti-hVEGFR2 antibodies and the fragments thereof provided herein further comprise a constant region of human IgG1, IgG2, IgG3, or IgG4. In certain embodiments, the anti-hVEGFR2 antibodies and antigen-binding fragments thereof provided herein comprises a constant region of IgG1 isotype. In certain embodiments, the constant region of human IgG1 comprises SEQ ID NO: 38

(ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF-PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE-PKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPE-VKFNWYVDGVEVHNAKTKPREEQYN-STYRVVSVLTVLHQDWLNGKE YKCKVSNKALPA-PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNG QPEN-NYKTTPPVLDSDGSFFLYSK-LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL-SPGK), or a homologous sequence having at least 80% (e.g. at least 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity thereof.

[0242] Constant region of IgG1 isotype can induce effector functions such as ADCC or CDC. Effector functions of the anti-hVEGFR2 antibodies and the antigen-binding fragments thereof provided herein can lead to cytotoxicity to cells expressing hVEGFR2. Effector functions can be evaluated using various assays such as Fc receptor binding assay, C1q binding assay, and cell lysis assay, and any of the assays described above for determining ADCC or CDC.

Antibody Variants

[0243] The anti-hVEGFR2 antibodies and antigen-binding fragments thereof provided herein also encompass various types of variants of the antibody sequences provided herein.

[0244] In certain embodiments, the variants comprise one or more modification(s) or substitution(s) in 1, 2, or 3 CDR sequences as provided in Table 1, in one or more FR sequences, in the heavy or light chain variable region sequences provided herein, and/or in the constant region

(e.g., Fc region). Such antibody variants retain specific binding affinity to hVEGFR2 of their parent antibodies, but have one or more desirable properties conferred by the modification(s) or substitution(s). For example, the antibody variants may have improved antigen-binding affinity, improved glycosylation pattern, reduced risk of glycosylation, reduced deamination, reduced or increased effector function(s), improved FcRn receptor binding, increased pharmacokinetic half-life, pH sensitivity, and/or compatibility to conjugation (e.g., one or more introduced cysteine residues), to name a few.

[0245] A parent antibody sequence may be screened to identify suitable or preferred residues to be modified or substituted, using methods known in the art, for example "alanine scanning mutagenesis" (see, for example, Cunningham and Wells (1989) Science, 244:1081-1085). Briefly, target residues (e.g., charged residues such as Arg, Asp, His, Lys, and Glu) can be identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine), and the modified antibodies are produced and screened for the interested property. If substitution at a particular amino acid location demonstrates an interested functional change, then the position can be identified as a potential residue for modification or substitution. The potential residues may be further assessed by substituting with a different type of residue (e.g., cysteine residue, positively charged residue, etc.).

1. Affinity Variant

[0246] An affinity variant retain specific binding affinity to hVEGFR2 of the parent antibody, or even have improved hVEGFR2 specific binding affinity over the parent antibody. Various methods known in the art can be used to achieve this purpose. For example, a library of antibody variants (such as Fab or scFv variants) can be generated and expressed with phage display technology, and then screened for the binding affinity to hVEGFR2. For another example, computer software can be used to virtually simulate the binding of the antibodies to hVEGFR2, and identify the amino acid residues on the antibodies which form the binding interface. Such residues may be either avoided in the substitution so as to prevent reduction in binding affinity, or targeted for substitution to provide for a stronger binding.

[0247] In certain embodiments, at least one (or all) of the substitution(s) in the CDR sequences, FR sequences, or variable region sequences comprises a conservative substitution. A "conservative substitution" with reference to amino acid sequence refers to replacing an amino acid residue with a different amino acid residue having a side chain with similar physiochemical properties. For example, conservative substitutions can be made among amino acid residues with hydrophobic side chains (e.g., Met, Ala, Val, Leu, and Ile), among residues with neutral hydrophilic side chains (e.g., Cys, Ser, Thr, Asn and Gln), among residues with acidic side chains (e.g., Asp, Glu), among amino acids with basic side chains (e.g., His, Lys, and Arg), or among residues with aromatic side chains (e.g., Trp, Tyr, and Phe). As known in the art, conservative substitution usually does not cause significant change in the protein conformational structure, and therefore could retain the biological activity of a protein.

[0248] In certain embodiments, the antibody or antigen-binding fragment provided herein comprises one or more amino acid residue substitutions in one or more CDR sequences, and/or one or more FR sequences. In certain embodiments, an affinity variant comprises no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 substitutions in one or more of the CDR sequences and/or FR sequences in total.

[0249] In certain embodiments, the anti-hVEGFR2 antibodies and antigen-binding fragments thereof comprise 1, 2, or 3 CDR sequences having at least 80% (e.g., at least 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%) sequence identity to that (or those) listed in Table 1, and in the meantime retain the binding affinity to hVEGFR2 at a level similar to or even higher than its parental antibody.

[0250] In certain embodiments, the anti-hVEGFR2 antibodies and antigen-binding fragments thereof comprise one or more variable region sequences having at least 80% (e.g., at least 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%) sequence identity to that (or those) of SEQ ID NOs: 50-63, 93-94 and 96-98, and in the meantime retain the binding affinity to hVEGFR2 at a level similar to or even higher than its parent antibody. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted, or deleted in a sequence selected from SEQ ID NOs: 50-63, 93-94 and 96-98. In some embodiments, the substitutions, insertions, or deletions occur in regions outside the CDRs (i.e., in the FRs).

2. Glycosylation Variant

[0251] The anti-hVEGFR2 antibodies and antigen-binding fragments provided herein also encompass a glycosylation variant, which can be obtained to either increase or decrease the extent of glycosylation of the antibody or antigen binding fragment. The term "glycosylation" as used herein, refers to enzymatic process that attaches glycans such as fucose, xylose, mannose, or GlcNAc phosphoserine glycan to proteins, lipids, or other organic molecules. Depending on the carbon linked to the glycan, glycosylation can be divided into five classes including: N-linked glycosylation, O-linked glycosylation, phospho-glycosylation, C-linked glycosylation, and glypiation.

[0252] Glycosylation of antibodies is typically N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue, for example, an asparagine residue in a tripeptide sequence such as asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly to serine or threonine.

[0253] In certain embodiments, the antibody or antigen-binding fragment thereof provided herein is afucosylated. The term "afucosylation," or "afucosylated," refers to the reduced or eliminated core-fucose on the N-glycan attached to the antibody. The majority glycans of human IgG antibodies are known as G0, G1 and G2, which are complex biantennary molecules with core fucose residue carrying zero, one or two terminal galactose.

[0254] Afucosylated antibody variants can be made using methods known in the art, for example, as described in US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. J. Mol. Biol. 336:1239-1249 (2004); Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004).

[0255] In certain embodiments, the antibody glycosylation variants can be obtained by, for example, removal of a native glycosylation site (e.g. by N297A substitution), such that tripeptide sequences for N-linked glycosylation sites or serine or threonine residues for O-linked glycosylation sites no longer present in the antibody or Fc sequence. Alternatively, in certain embodiments, antibody glycosylation variants can be obtained by producing the antibody in a host cell line that is defective in adding the selected sugar group(s) to the mature core carbohydrate structure in the antibody.

3. Cysteine-Engineered Variant

[0256] The anti-hVEGFR2 antibodies and antigen-binding fragments provided herein also encompass a cysteine-engineered variant, which comprises one or more introduced free cysteine amino acid residues.

[0257] A free cysteine residue is one which is not part of a disulfide bridge. A cysteine-engineered variant is useful for conjugation with, for example a cytotoxic and/or imaging compound, a label, or a radioisoptype among others, at the site of the engineered cysteine, through for example a maleimide or haloacetyl. Methods for engineering antibodies or antigen-binding fragments to introduce free cysteine residues are known in the art, see, for example, WO2006/034488.

Antigen-Binding Fragments

[0258] Provided herein are also anti-hVEGFR2 antigen-binding fragments. Various types of antigen-binding fragments are known in the art and can be developed based on the anti-hVEGFR2 antibodies provided herein, including for example, the exemplary antibodies whose CDR sequences are shown in Tables 1, and their different variants (such as affinity variants, glycosylation variants, Fc variants, cysteine-engineered variants and so on).

[0259] In certain embodiments, an anti-hVEGFR2 antigen-binding fragment provided herein is a diabody, a Fab, a Fab', a $F(ab')_2$, a Fd, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), an scFv dimer (bivalent diabody), a multispecific antibody, a camelized single domain antibody, a nanobody, a domain antibody, or a bivalent domain antibody.

[0260] Various techniques can be used for the production of such antigen-binding fragments. Illustrative methods include, enzymatic digestion of intact antibodies (see, e.g., Morimoto et al., Journal of Biochemical and Biophysical Methods 24:107-117 (1992); and Brennan et al., Science, 229:81 (1985)), recombinant expression by host cells such as E. Coli (e.g., for Fab, Fv and ScFv antibody fragments), screening from a phage display library as discussed above (e.g., for ScFv), and chemical coupling of two Fab'-SH fragments to form $F(ab')_2$ fragments (Carter et al., Bio/Technology 10:163-167 (1992)). Other techniques for the production of antibody fragments will be apparent to a skilled practitioner.

[0261] In certain embodiments, the antigen-binding fragment is a scFv. Generation of scFv is described in, for example, WO 93/16185; U.S. Pat. Nos. 5,571,894; and 5,587,458. scFv may be fused to an effector protein at either the amino or the carboxyl terminus to provide for a fusion protein (see, for example, Antibody Engineering, ed. Borrebaeck).

[0262] In certain embodiments, the anti-hVEGFR2 antibodies and antigen-binding fragments thereof provided herein are bivalent, tetravalent, hexavalent, or multivalent. The term "valent" as used herein refers to the presence of a specified number of antigen binding sites in a given molecule. As such, the terms "bivalent", "tetravalent", and "hexavalent" denote the presence of two binding site, four binding sites, and six binding sites, respectively, in an antigen-binding molecule. Any molecule being more than bivalent is considered multivalent, encompassing for example, trivalent, tetravalent, hexavalent, and so on.

[0263] A bivalent molecule can be monospecific if the two binding sites are both specific for binding to the same antigen or the same epitope. This, in certain embodiments, provides for stronger binding to the antigen or the epitope than a monovalent counterpart. Similar, a multivalent molecule may also be monospecific. In certain embodiments, in a bivalent or multivalent antigen-binding moiety, the first valent of binding site and the second valent of binding site are structurally identical (i.e. having the same sequences), or structurally different (i.e. having different sequences albeit with the same specificity).

[0264] A bivalent can also be bispecific, if the two binding sites are specific for different antigens or epitopes. This also applies to a multivalent molecule. For example, a trivalent molecule can be bispecific when two binding sites are monospecific for a first antigen (or epitope) and the third binding site is specific for a second antigen (or epitope).

Bispecific Antibodies

[0265] In certain embodiments, the antibodies and antigen-binding fragments thereof provided herein are bispecific. The term "bispecific" as used herein encompasses molecules having more than two specificity and molecules having more than two specificity, i.e. multispecific. In certain embodiments, the bispecific antibodies and antigen-binding fragments thereof provided herein is capable of specifically binding to a first and a second epitopes of hVEGFR2, or capable of specifically binding to hVEGFR2 and a second antigen. In certain embodiments, the first epitope and the second epitopes of hVEGFR2 are distinct from each other or non-overlapping. In certain embodiments, the bispecific antibodies and antigen-binding fragments thereof can bind to both the first epitope and the second epitope at the same time.

[0266] In certain embodiments, the bispecific antibody comprises a first binding domain and a second binding domain, wherein the first binding domain comprises a HCDR1 comprising the sequence selected from the group consisting of SEQ ID NOs: 25, 31, 34 and 1, a HCDR2 comprising the sequence selected from the group consisting of SEQ ID NOs: 26, 32, 35 and 2, a HCDR3 comprising the sequence selected from the group consisting of SEQ ID NOs: 27, 33, 36 and 3, a LCDR1 comprising the sequence selected from the group consisting of SEQ ID NOs: 28 and 4, a LCDR2 comprising the sequence selected from the group consisting of SEQ ID NOs: 29 and 5, and a LCDR3 comprising the sequence selected from the group consisting of SEQ ID NOs: 30 and 6; and wherein the second binding domain comprises a HCDR1 comprising the sequence selected from the group consisting of SEQ ID NOs: 7, 13

and 19, a HCDR2 comprising the sequence selected from the group consisting of SEQ ID NOs: 8, 14 and 20, a HCDR3 comprising the sequence selected from the group consisting of SEQ ID NOs: 9, 15 and 21, a LCDR1 comprising the sequence selected from the group consisting of SEQ ID NOs: 10, 16 and 22, a LCDR2 comprising the sequence selected from the group consisting of SEQ ID NOs: 11, 17 and 23, and a LCDR3 comprising the sequence selected from the group consisting of SEQ ID NOs: 12, 18 and 24.

[0267] In certain embodiments, the bispecific antibody comprises a first binding domain and a second binding domain,

[0268] wherein the first binding domain comprising:

[0269] a) a HCDR1 comprises the sequence of SEQ ID NO: 25, a HCDR2 comprising the sequence of SEQ ID NO: 26, a HCDR3 comprising the sequence of SEQ ID NO: 27; a LCDR1 comprising the sequence of SEQ ID NO: 28, a LCDR2 comprising the sequence of SEQ ID NO: 29, and a LCDR3 comprising the sequence of SEQ ID NO: 30; or

[0270] b) a HCDR1 comprising the sequence of SEQ ID NO: 31, a HCDR2 comprising the sequence of SEQ ID NO: 32 or SEQ ID NO: 37, a HCDR3 comprising the sequence of SEQ ID NO: 33, a LCDR1 comprising the sequence of SEQ ID NO: 28, a LCDR2 comprising the sequence of SEQ ID NO: 29, and a LCDR3 comprising the sequence of SEQ ID NO: 30; or

[0271] c) a HCDR1 comprising the sequence of SEQ ID NO: 34, a HCDR2 comprising the sequence of SEQ ID NO: 35 or SEQ ID NO: 37, a HCDR3 comprising the sequence of SEQ ID NO: 36, a LCDR1 comprising the sequence of SEQ ID NO: 28, a LCDR2 comprising the sequence of SEQ ID NO: 29, and a LCDR3 comprising the sequence of SEQ ID NO: 30; or

[0272] d) a HCDR1 comprising the sequence of SEQ ID NO: 1, a HCDR2 comprising the sequence of SEQ ID NO: 2, a HCDR3 comprising the sequence of SEQ ID NO: 3, a LCDR1 comprising the sequence of SEQ ID NO: 4, a LCDR2 comprising the sequence of SEQ ID NO: 5, and a LCDR3 comprising the sequence of SEQ ID NO: 6; and wherein the second binding domain comprising:

[0273] e) a HCDR1 comprising the sequence of SEQ ID NO: 7, a HCDR2 comprising the sequence of SEQ ID NO: 8, a HCDR3 comprising the sequence of SEQ ID NO: 9; a LCDR1 comprising the sequence of SEQ ID NO: 10, a LCDR2 comprising the sequence of SEQ ID NO: 11, and a LCDR3 comprising the sequence of SEQ ID NO: 12; or

[0274] f) a HCDR1 comprising the sequence of SEQ ID NO: 13, a HCDR2 comprising the sequence of SEQ ID NO: 14, a HCDR3 comprising the sequence of SEQ ID NO: 15, a LCDR1 comprising the sequence of SEQ ID NO: 16, a LCDR2 comprising the sequence of SEQ ID NO: 17, and a LCDR3 comprising the sequence of SEQ ID NO: 18, or

[0275] g) a HCDR1 comprising the sequence of SEQ ID NO: 19, a HCDR2 comprising the sequence of SEQ ID NO: 20, a HCDR3 comprising the sequence of SEQ ID NO: 21, a LCDR1 comprising the sequence of SEQ ID NO: 22, a LCDR2 comprising the sequence of SEQ ID NO: 23, and a LCDR3 comprising the sequence of SEQ ID NO: 24.

[0276] In certain embodiments, the second antigen is different from hVEGFR2.

[0277] In certain embodiments, the second antigen is an immune related target. In some embodiments, the bispecific antibodies and antigen-binding fragments thereof specifically bind to hVEGFR2 and an immune related target, and are capable of targeting the immune cells to hVEGFR2-expressing cells (e.g. hVEGFR2-expressing tumor cells), and/or activating hVEGFR2 specific immune response to the hVEGFR2-expressing target cells. An immune related target as used herein, encompasses a biological molecule that is involved in the generation or modulation of an immune response, optionally, cellular immune responses. An example of the immune related target is immune checkpoint molecule, and a surface molecule of a cytolytic immune cell such as T cell or natural killer (NK) cell.

[0278] Immune checkpoint molecule can mediate co-stimulatory signal to augment immune response, or can mediate co-inhibitory signals to suppress immune response. Examples of an immune checkpoint molecule include, for example, PD-L1, PD-L2, PD-1, CLTA-4, TIM-3, LAG3, A2AR, CD160, 2B4, TGF β, VISTA, BTLA, TIGIT, LAIR1, OX40, CD2, CD27, CD28, CD30, CD40, CD122, ICAM-1, IDO, NKG2C, SLAMF7, SIGLEC7, NKp80, CD160, B7-H3, LFA-1, 1COS, 4-1BB, GITR, BAFFR, HVEM, CD7, LIGHT, IL-2, IL-15, CD3, CD16 and CD83.

[0279] Cytolytic immune cells can be triggered by its surface molecule to attack and mediate lysis of a target cell such as a tumor cell. In certain embodiments, the second antigen is a T cell surface antigen. Examples of a T cell surface antigen include, without limitation, an antigen selected from the group consisting of CD3, CD2, CD4, CD5, CD6, CD8, CD28, CD40L and/or CD44, preferably CD3. In certain embodiments, said second antigen is the epsilon-chain of CD3. In certain embodiments, binding of said bispecific antibody to CD3 on T cells results in proliferation and/or activation of said T cells, which induces release of cytotoxic factors, e.g. perforins and granzymes, and cytolysis and apoptosis of the target cells. In certain embodiments, the second antigen is a NK cell surface antigen, such as CD16 (FcγRIII) or CD56. In certain embodiments, binding of bispecific antibody to CD16 on NK cells leads to NK-cell degranulation and perforin-dependent target cell lysis (ADCC) of the target cells.

[0280] In certain embodiments, the second antigen comprises a tumor antigen. "Tumor antigen" as used herein refers to tumor specific antigens (e.g. those unique to tumor cells and normally not found on non-tumor cells), tumor-associated antigens (e.g. found in both tumor and non-tumor cells but expressed differently in tumor cells), and tumor neo-antigens (e.g. that are expressed in cancer cells because of somatic mutations that change the protein sequence or create fusion proteins between two unrelated sequences).

[0281] Examples of tumor antigens include, without limitation, EpCAM, HER2/neu, HER3/neu, C250, CEA, MAGE, proteoglycans, VEGF, EGFR, αVβ3-integrin, HLA, HLA-DR, ASC, CD1, CD2, CD4, CD6, CD7, CD8, CD11, CD13, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD30, CD33, CD37, CD40, CD41, CD47, CD52, c-erb-2, CALLA, MHCII, CD44v3, CD44v6, p97, ganglioside GM1, GM2, GM3, GDla, GD1b, GD2, GD3, GT1 b, GT3, GQ1, NY-ESO-1, NFX2, SSX2, SSX4, Trp2, gp100 (Pmel 17), tyrosinase, Muc-1, telomerase, survivin, G250, p53, CA125 MUC, Wue antigen, Lewis Y antigen, HSP-27,

HSP-70, HSP-72, HSP-90, Pgp, MCSP, EpHA2 and cell surface targets GC1 82, GT468 or GT512, PD-L1, arboviral E protein epitope, glioma-associated antigen, carcinoembryonic antigen (CEA), 3-human chorionic gonadotropin, alphafetoprotein (AFP), lectin-reactive AFP, thyroglobulm, RAGE-1, MN-CA IX, human telomerase reverse transcriptase, RU1, RU2 (AS), intestinal carboxyi esterase, mut hsp70-2, M-CSF, prostase, prostate-specific antigen (PSA), PAP, NY-ESO-1, LAGE-la, p53, prostein, PSMA, survivin and telomerase, prostate-carcinoma tumor antigen-1 (PCTA-1), MAGE, ELF2M, neutrophil elastase, ephrinB2, CD22, insulin growth factor (IGF)-I, IGF-II, IGF-I receptor and mesothelin, ART-1/MelanA (MART-1), tyrosinase, TRP-1, TRP-2 and tumor-specific multilineage antigens such as MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, pi 5; Ras, unique tumor antigens resulting from chromosomal translocations; such as BCR-ABL, E2A-PRL, H4-RET, 1GH-IGK, MYL-RAR; and viral antigens, such as the Epstein Barr virus antigens EBVA and the human papillomavirus (HPV) antigens E6 and E7; protein-based antigens include TSP-180, MAGE-4, MAGE-5, MAGE-6, RAGE, NY-ESO, pl 85erbB2, pl 80erbB-3, c-met, nm-23H 1, PSA, TAG-72, CA19-9, CA72-4, CAM 17.1, NuMa, K-ras, beta-Catenin, CDK4, Mum-1, p15, p16, 43-9F, 5T4(791Tgp72), a-fetoprotem, beta-HCG, BCA225, BTAA, CA 125, CA 15-3CA 27.29BCAA, CA 195, CA 242, CA-50, CAM43, CD68I, CO-029, FGF-5, G250, Ga733VEpCAM, HTgp-175, M344, MA-50, MG7-Ag, MOV 18, NB/70K, NY-CO-1, RCAS 1, SDCCAG16, TA-90Mac-2 binding protein, cyclophilin C-associated protein, TAAL6, TAG72, TLP, and TPS.

[0282] Bispecific antibodies and antigen-binding fragments thereof provided herein can be in a suitable format known in the art. For example, an exemplary bispecific format can be, bispecific diabodies, scFv-based bispecific formats, IgG-scFv fusions, dual variable domain (DVD)-Ig, Quadroma, knobs-into-holes, common light chain (e.g., common light chain with knobs-into-holes, etc.), BiTE, CrossMab, CrossFab, Duobody, SEEDbody, leucine zipper, dual acting Fab (DAF)-IgG, and Mab^2 bispecific formats (see, e.g., Brinkmann et al. 2017, Mabs, 9(2): 182-212). The bispecific molecules can be in symmetric or asymmetric architecture.

[0283] The bispecific antibodies and antigen-binding fragments provided herein can be made with any suitable methods known in the art.

[0284] In one embodiment, two immunoglobulin heavy chain-light chain pairs having different antigenic specificities are co-expressed in a host cell to produce bispecific antibodies in a recombinant way (see, for example, Milstein and Cuello, Nature, 305: 537 (1983)), followed by purification by affinity chromatography.

[0285] In another embodiment, sequences encoding the antibody heavy chain variable domains for the two specificities are respectively fused to immunoglobulin constant domain sequences, followed by insertion to one or more expression vector(s) which is/are co-transfected with an expression vector for the light chain sequences to a suitable host cell for recombinant expression of the bispecific antibody (see, for example, WO 94/04690; Suresh et al., Methods in Enzymology, 121:210 (1986)). Similarly, scFv dimers can also be recombinantly constructed and expressed from a host cell (see, e.g., Gruber et al., J. Immunol., 152:5368 (1994).)

[0286] In another method, leucine zipper peptides from the Fos and Jun. proteins can be linked to the Fab' portions of two different antibodies by gene fusion. The linked antibodies are reduced at the hinge region to four half antibodies (i.e. monomers) and then re-oxidized to form heterodimers (Kostelny et al., J. Immunol., 148(5):1547-1553 (1992)).

[0287] The two antigen-binding domains may also be conjugated or cross-linked to form a bispecific antibody or antigen-binding fragment. For example, one antibody can be coupled to biotin while the other antibody to avidin, and the strong association between biotin and avidin would complex the two antibodies together to form a bispecific antibody (see, for example, U.S. Pat. No. 4,676,980; WO 91/00360, WO 92/00373, and EP 03089). For another example, the two antibodies or antigen-binding fragments can be cross-linked by conventional methods known in the art, for example, as disclosed in U.S. Pat. No. 4,676,980.

[0288] Bispecific antigen-binding fragments may be generated from a bispecific antibody, for example, by proteolytic cleavage, or by chemical linking. For example, an antigen-binding fragment (e.g., Fab') of an antibody may be prepared and converted to Fab'-thiol derivative and then mixed and reacted with another converted Fab' derivative having a different antigenic specificity to form a bispecific antigen-binding fragment (see, for example, Brennan et al., Science, 229: 81 (1985)).

[0289] In certain embodiments, the bispecific antibody or antigen-binding fragments thereof provided herein may be engineered at the interface so that a knob-into-hole association can be formed to promote heterodimerization of the two different antigen-binding sites. This can maximize the percentage of heterodimers which are recovered from recombinant cell culture. "Knob-into-hole" as used herein, refers to an interaction between two polypeptides (such as Fc), where one polypeptide has a protuberance (i.e. "knob") due to presence of an amino acid residue having a bulky side chain (e.g., tyrosine or tryptophan), and the other polypeptide has a cavity (i.e. "hole") where a small side chain amino acid residue resides (e.g., alanine or threonine), and the protuberance is positionable in the cavity so as to promote interaction of the two polypeptides to form a heterodimer or a complex. Methods of generating polypeptides with knobs-into-holes are known in the art, e.g., as described in U.S. Pat. No. 5,731,168.

Conjugates

[0290] In some embodiments, the anti-hVEGFR2 antibodies and antigen-binding fragments thereof are linked to one or more conjugate moieties. A conjugate is a moiety that can be attached to the antibody or antigen-binding fragment thereof. It is contemplated that a variety of conjugates may be linked to the antibodies or antigen-binding fragments provided herein (see, for example, "Conjugate Vaccines", Contributions to Microbiology and Immunology, J. M. Cruse and R. E. Lewis, Jr. (eds.), Carger Press, New York, (1989)). These conjugates may be linked to the antibodies or antigen-binding fragments by covalent binding, affinity binding, intercalation, coordinate binding, complexation, association, blending, or addition, among other methods. In certain embodiments, the antibodies or antigen binding fragments thereof are linked to one or more conjugates via a linker. In certain embodiments, the linker is a hydrazone

linker, a disulfide linker, a bifunctional linker, dipeptide linker, glucuronide linker, a thioether linker.

[0291] In certain embodiments, the anti-hVEGFR2 antibodies and antigen-binding fragments disclosed herein may be engineered to contain specific sites outside the epitope binding portion that may be utilized for binding to one or more conjugates. For example, such a site may include one or more reactive amino acid residues, such as for example cysteine or histidine residues, to facilitate covalent linkage to a conjugate.

[0292] The conjugate can be a clearance-modifying agent, therapeutic agent (e.g., a chemotherapeutic agent), a toxin, a radioactive isotope, a detectable label (e.g., a lanthanide, a luminescent label, a fluorescent label, or an enzyme-substrate label), a pharmacokinetic modifying moiety, a DNA-alkylators, a topoisomerase inhibitor, a tubulin-binders, other anticancer drugs, or a purifying moiety (such as a magnetic bead or nanoparticle).

[0293] Examples of detectable label may include a fluorescent labels (e.g., fluorescein, rhodamine, dansyl, phycoerythrin, or Texas Red), enzyme-substrate labels (e.g., horseradish peroxidase, alkaline phosphatase, luceriferases, glucoamylase, lysozyme, saccharide oxidases or P-D-galactosidase), radioisotuopes, other lanthanides, luminescent labels, chromophoric moiety, digoxigenin, biotin/avidin, a DNA molecule or gold for detection.

[0294] Examples of radioisotopes may include ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{35}S, ^{3}H, ^{111}In, ^{112}In, ^{14}C, ^{64}Cu, ^{67}Cu, ^{86}Y, ^{88}Y, ^{90}Y, ^{177}Lu, ^{211}At, ^{186}Re, ^{188}Re, ^{153}Sm, ^{212}Bi, and ^{32}P. Radioisotope labelled antibodies are useful in receptor targeted imaging experiments.

[0295] In certain embodiments, the conjugate can be a pharmacokinetic modifying moiety such as PEG which helps increase half-life of the antibody. Other suitable polymers include, such as, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, copolymers of ethylene glycol/propylene glycol, and the like.

[0296] In certain embodiments, the conjugate can be a purification moiety such as a magnetic bead or a nanoparticle.

Antibody-Drug Conjugates

[0297] In certain embodiments, the present disclosure provides antibody-drug conjugates (ADC) comprising any of the above anti-hVEGFR2 antibodies or antigen-binding fragments conjugated to a cytotoxic agent.

[0298] ADC can be useful for local delivery of cytotoxic agents, for example, in the treatment of cancer. This allows for targeted delivery of cytotoxic agents to tumors and intracellular accumulation therein, which is particularly useful where systemic administration of these unconjugated cytotoxic agents may result in unacceptable levels of toxicity to normal cells as well as the tumor cells sought to be eliminated (Baldwin et al., (1986) Lancet pp. (Mar. 15, 1986):603-05; Thorpe, (1985) "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in Monoclonal Antibodies '84: Biological And Clinical Applications, A. Pinchera et al. (ed.s), pp. 475-506; Syrigos and Epenetos (1999) Anticancer Research 19:605-614; Niculescu-Duvaz and Springer (1997) Adv. Drg Del. Rev. 26:151-172; U.S. Pat. No. 4,975,278).

[0299] In certain embodiments, the cytotoxic agent can be any agent that is detrimental to cells or that can damage or kill cells. In certain embodiments, the cytotoxic agent is optionally a toxin, a chemotherapeutic agent (such as a DNA-alkylators, a topoisomerase inhibitor, a tubulin-binders, a growth inhibitory agent, or other anticancer drugs), or a radioactive isotope.

[0300] Examples of toxins include bacterial toxins and plant toxins, such as for example, diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin, abrin, modeccin, alpha-sarcin, *Aleurites fordii*. proteins, dianthin proteins, *Phytolaca americana* proteins (PARI, PAPII, and PAP-S), *Momordica charantia* inhibitor, curcin, crotin, *Sapaonaria officinalis* inhibitor, gelonin, restrictocin, phenomycin, enomycin, and the tricothecenes (see, e.g., WO 93/21232). Such a large molecule toxin can be conjugated to the antibodies or antigen-binding fragments provided herein using methods known in the art, for example, as described in Vitetta et al (1987) Science, 238:1098.

[0301] The cytotoxic agent can also be small molecule toxins and chemotherapeutic agents, such as geldanamycin (Mandler et al (2000) Jour. of the Nat. Cancer Inst. 92(19): 1573-1581; Mandler et al (2002) Bioconjugate Chem. 13:786-791), maytansine and maytansinoids (EP 1391213; Liu et al., (1996) Proc. Natl. Acad. Sci. USA 93:8618-8623; U.S. Pat. No. 5,208,020), calicheam icin (Lode et al (1998) Cancer Res. 58:2928; Hinman et al (1993) Cancer Res. 53:3336-3342), taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, vindesine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin and analogs thereof, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine), calicheamicin, maytansinoids, dolastatins, auristatins such as MMAE and MMAF (U.S. Pat. Nos. 5,635,483; 5,780,588), dolostatins, a trichothecene, and CC1065, and the derivatives thereof having cytotoxic activity.

[0302] The cytotoxic agent can also be a highly radioactive isotope. Examples include At ^{211} I^{131}, I^{125}, Y^{90}, Re^{186}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu. Methods of conjugation of a radioisotope to an antibody is known in the art, for example, via a suitable ligand reagent (see, e.g., WO94/11026; Current Protocols in Immunology, Volumes 1 and 2, Coligen et al, Ed. Wiley-Interscience, New York, N.Y, Pubs. (1991)). A ligand reagent has a chelating ligand that can bind, chelate or otherwise complex a radioisotope metal, and also has a functional group that is reactive with a thiol of cysteine of an antibody or antigen-binding fragment. Exemplary chelating ligands include DOTA, DOTP, DOTMA, DTPA and TETA (Macrocyclics, Dallas, Tex.).

[0303] The cytotoxic agents can be linked to an antibody or antigen-binding fragment via any suitable linkers known in the art, see, for example, in U.S. Pat. Nos. 5,208,020, 6,441,163, or EP Patent 0 425 235 B1, Chari et al., Cancer

Research 52:127-131 (1992), and US 2005/0169933 A1, the disclosures of which are hereby expressly incorporated by reference.

[0304] In certain embodiments, the linker is cleavable under a particular physiological environment, thereby facilitating release of the cytotoxic drug in the cell. For example, the linker can be an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker, thioether linker, and esterase labile linker (Chari et al., Cancer Research 52:127-131(1992); U.S. Pat. No. 5,208,020). In some embodiments, the linker may comprise amino acid residues, such as a dipeptide, a tripeptide, a tetrapeptide or a pentapeptide. The amino acid residues in the linker may be natural or non-naturally occurring amino acid residues. Examples of such linkers include: valine-citrulline (ve or val-cit), alanine-phenylalanine (af or ala-phe), glycine-valine-citrulline (gly-yal-cit), glycine-glycine-glycine (gly-gly-gly), an valine-citrullin-p-aminobenzyloxycaronyl ("vc-PAB"). Amino acid linker components can be designed and optimized in their selectivity for enzymatic cleavage by a particular enzymes, for example, a tumor-associated protease, cathepsin B, C and D, or a plasmin protease.

[0305] In certain embodiments, the cytotoxic agents can be linked to the antibody or antigen-binding fragment thereof provided herein by a bifunctional linker reagent include, such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), bis-active fluorine compounds (such as 1,5-difluom-2,4-dinitrobenzene), BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPRH, SBAP, SIA, SIAB, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSG (succinimidyl-(4-vinylsulfone)benzoate). Those linker reagents are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, Ill., USA, see pages 467-498, 2003-2004 Applications Handbook and Catalog).

[0306] In certain embodiments, in the ADC provided herein, an antibody (or antigen-binding fragment thereof) is conjugated to one or more cytotoxic agents at an antibody: agent ratio of about 1 to about 20, about 1 to about 6, about 2 to about 6, about 3 to about 6, about 2 to about 5, about 2 to about 4, or about 3 to about 4.

[0307] The ADC provided herein may be prepared by any suitable methods known in the art. In certain embodiments, a nucleophilic group of the antibody (or antigen-binding fragment thereof) is first reacted with a bifunctional linker reagent and then linked to the cytotoxic agent, or the other way around, i.e., first reacting a nucleophilic of the cytotoxic agent with a bifunctional linker and then linking to the antibody.

[0308] In certain embodiments, the cytotoxic agent may contain (or modified to contain) a thiol reactive functional group which may react with a cysteine thiol of a free cysteine of the antibodies or antigen-binding fragments provided herein. Exemplary thiol-reactive functional group include, for example, a maleimide, an iodoacetamide, a pyridyl disulfide, haloacetyl, succinimidyl ester (e.g., NHS, N-hydroxysuccinimide), isothiocyanate, sulfonyl chloride, 2,6-dichlorotriazinyl, pentafluorophenyl ester, or phosphoramidite (Haugland, 2003, Molecular Probes Handbook of Fluorescent Probes and Research Chemicals, Molecular Probes, Inc.; Brinkley, 1992, Bioconjugate Chem. 3:2; Garman, 1997, Non-Radioactive Labelling: A Practical Approach, Academic Press, London; Means (1990) Bioconjugate Chem. 1:2; Hermanson, G. in Bioconjugate Techniques (1996) Academic Press, San Diego, pp. 40-55, 643-671).

[0309] The cytotoxic agent or the antibody may react with a linking reagent before being conjugated to form the ADC. For example, N-hydroxysuccinimidyl ester (NHS) of a cytotoxic agent may be performed, isolated, purified, and/or characterized, or it may be formed in situ and reacted with a nucleophilic group of an antibody. Typically, the carboxyl form of the conjugate is activated by reacting with some combination of a carbodiimide reagent, e.g., dicyclohexylcarbodiimide; diisopropyl carbodiimide, or a uronium reagent, e.g., TsTu (O-(N-Succinimidyl)-N,N,N',N'-tetramethyluronium tetrafluoroborate, HBTU (O-benzotriazol-1-yl)-N,N,N'N'-tetramethyluronium hexafluorophosphate), or HATU (0-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate), an activator, such as 1-hydroxybenzotriazole (HOBt), and N-hydroxysuccinimide to give the NHS ester. In some cases, the cytotoxic agent and the antibody may be linked by in situ activation and reaction to form the ADC in one step. Other activating and linking reagents include TBTU (2-(1H-benzotriazo-1-yl)-1-1,3,3-tetramethyluronium hexafluorophosphate), TFFH (N,N',N'', N'''-tetramethyluronium 2-fluoro-hexafluorophosphate), PyBOP (benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate, EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydro-quinoline), DCC (dicyclohexylcarbodiimide); DIPCDI (diisopropylcarbodiimide), MSNT (1-(mesitylene-2-sulfonyl)-3-nitro-1H-1,2,4-triazole, and aryl sulfonyl halides, e.g., triisopropylbenzenesulfonyl chloride. In another example, the antibody or antigen-binding fragments may be conjugated to biotin, then indirectly conjugated to a second conjugate that is conjugated to avidin.

Polynucleotides and Recombinant Methods

[0310] The present disclosure provides isolated polynucleotides that encode the anti-hVEGFR2 antibodies and antigen-binding fragments thereof. The term "nucleic acid" or "polynucleotide" as used herein refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless otherwise indicated, a particular polynucleotide sequence also implicitly encompasses conservatively modified variants thereof (e.g. degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (see Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); and Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)).

[0311] DNA encoding the monoclonal antibody is readily isolated and sequenced using conventional procedures (e.g.

by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). The encoding DNA may also be obtained by synthetic methods.

[0312] The present disclosure provides vectors (e.g. expression vectors) comprising the isolated polynucleotide provided herein. In certain embodiments, the expression vector provided herein comprises the polynucleotide encoding the antibodies or antigen-binding fragments thereof provided herein, at least one promoter (e.g. SV40, CMV, EF-la) operably linked to the polynucleotide sequence, and at least one selection marker. Examples of vectors include, but are not limited to, retrovirus (including lentivirus), adenovirus, adeno-associated virus, herpesvirus (e.g. herpes simplex virus), poxvirus, baculovirus, papillomavirus, papovavirus (e.g. SV40), lambda phage, and M13 phage, plasmids such as pcDNA3.3, pMD18-T, pOptivec, pCMV, pEGFP, pIRES, pQD-Hyg-GSeu, pALTER, pBAD, pcDNA, pCal, pL, pET, pGEMEX, pGEX, pCI, pEGFT, pSV2, pFUSE, pVITRO, pVIVO, pMAL, pMONO, pSELECT, pUNO, pDUO, Psg5L, pBABE, pWPXL, pBI, p15TV-L, pProl8, pTD, pRS10, pLexA, pACT2.2, pCMV-SCRIPT. RTM., pCDM8, pCDNA1.1/amp, pcDNA3.1, pRc/RSV, PCR 2.1, pEF-1, pFB, pSG5, pXT1, pCDEF3, pSVSPORT, pEF-Bos etc.

[0313] Vectors comprising the polynucleotide sequence encoding the antibody or antigen-binding fragment thereof can be introduced to a host cell for cloning or gene expression. Suitable host cells for cloning or expressing the DNA in the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *Escherichia*, e.g. *E. coli, Enterobacter, Erwinia, Klebsiella, Proteus, Salmonella*, e.g. *Salmonella typhimurium, Serratia*, e.g. *Serratia marcescans*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis, Pseudomonas* such as *P. aeruginosa*, and *Streptomyces.*

[0314] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for anti-hVEGFR2 antibody-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe; Kluyveromyces* hosts such as, e.g. *K. lactis, K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickeramii* (ATCC 24,178), *K. waltii* (ATCC 56,500), K. *drosophilarum* (ATCC 36,906), *K. thermotolerans*, and *K. marxianus; yarrowia* (EP 402, 226); *Pichia pastoris* (EP 183,070); *Candida; Trichoderma* reesia (EP 244,234); *Neurospora crassa; Schwanniomyces* such as *Schwanniomyces occidentalis*; and filamentous fungi such as, e.g. *Neurospora, Penicillium, Tolypocladium*, and *Aspergillus* hosts such as *A. nidulans* and *A. niger.*

[0315] Suitable host cells for the expression of glycosylated antibodies or antigen-fragment provided herein are derived from multicellular organisms such as invertebrate cells, for example plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as Spodopterafrugiperda (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruiffly), and *Bombyx mori* have been identified. A variety of viral strains for transfection are publicly available, e.g. the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of Spodopterafrugiperda cells. Plant cell cultures of cotton, corn, potato, soybean, *petunia*, tomato, and tobacco can also be utilized as hosts.

[0316] However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); mouse sertoli cells (TM4, *Mather, Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2). In some preferable embodiments, the host cell is a mammalian cultured cell line, such as CHO, BHK, NS0, 293 and their derivatives. In some preferable embodiments, the host cell is CHO and its derivatives.

[0317] Host cells are transformed with the above-described expression or cloning vectors for anti-hVEGFR2 antibody production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. In another embodiment, the antibody may be produced by homologous recombination known in the art.

[0318] The host cells used to produce the antibodies or antigen-binding fragments provided herein may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium (MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium (DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham et al., Meth. Enz. 58:44 (1979), Barnes et al., Anal. Biochem. 102:255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Pat. Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously

used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

[0319] When using recombinant techniques, the antibody can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration. Carter et al., Bio/Technology 10:163-167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic space of *E. coli*. Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) over about 30 min. Cell debris can be removed by centrifugation. Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

[0320] The anti-hVEGFR2 antibodies and antigen-binding fragments thereof prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, DEAE-cellulose ion exchange chromatography, ammonium sulfate precipitation, salting out, and affinity chromatography, with affinity chromatography being the preferred purification technique.

[0321] In certain embodiments, Protein A immobilized on a solid phase is used for immunoaffinity purification of the antibody and antigen-binding fragment thereof. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human gamma1, gamma2, or gamma4 heavy chains (Lindmark et al., J. Immunol. Meth. 62:1-13 (1983)).

[0322] Protein G is recommended for all mouse isotypes and for human gamma3 (Guss et al., EMBO J. 5:1567 1575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a CH3 domain, the Bakerbond ABX.TM. resin (J. T. Baker, Phillipsburg, N.J.) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

[0323] Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, preferably performed at low salt concentrations (e.g., from about 0-0.25M salt).

Pharmaceutical Composition

[0324] The present disclosure further provides pharmaceutical compositions comprising the anti-hVEGFR2 antibodies or antigen-binding fragments thereof and one or more pharmaceutically acceptable carriers.

[0325] Pharmaceutical acceptable carriers for use in the pharmaceutical compositions disclosed herein may include, for example, pharmaceutically acceptable liquid, gel, or solid carriers, aqueous vehicles, nonaqueous vehicles, antimicrobial agents, isotonic agents, buffers, antioxidants, anesthetics, suspending/dispending agents, sequestering or chelating agents, diluents, adjuvants, excipients, or non-toxic auxiliary substances, other components known in the art, or various combinations thereof.

[0326] Suitable components may include, for example, antioxidants, fillers, binders, disintegrants, buffers, preservatives, lubricants, flavorings, thickeners, coloring agents, emulsifiers or stabilizers such as sugars and cyclodextrins. Suitable antioxidants may include, for example, methionine, ascorbic acid, EDTA, sodium thiosulfate, platinum, catalase, citric acid, cysteine, thioglycerol, thioglycolic acid, thiosorbitol, butylated hydroxanisol, butylated hydroxytoluene, and/or propyl gallate. As disclosed herein, inclusion of one or more antioxidants such as methionine in a composition comprising an antibody or antigen-binding fragment and conjugates as provided herein decreases oxidation of the antibody or antigen-binding fragment. This reduction in oxidation prevents or reduces loss of binding affinity, thereby improving antibody stability and maximizing shelf-life. Therefore, in certain embodiments compositions are provided that comprise one or more antibodies or antigen-binding fragments as disclosed herein and one or more antioxidants such as methionine. Further provided are methods for preventing oxidation of, extending the shelf-life of, and/or improving the efficacy of an antibody or antigen-binding fragment as provided herein by mixing the antibody or antigen-binding fragment with one or more antioxidants such as methionine.

[0327] To further illustrate, pharmaceutical acceptable carriers may include, for example, aqueous vehicles such as sodium chloride injection, Ringer's injection, isotonic dextrose injection, sterile water injection, or dextrose and lactated Ringer's injection, nonaqueous vehicles such as fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil, or peanut oil, antimicrobial agents at bacteriostatic or fungistatic concentrations, isotonic agents such as sodium chloride or dextrose, buffers such as phosphate or citrate buffers, antioxidants such as sodium bisulfate, local anesthetics such as procaine hydrochloride, suspending and dispersing agents such as sodium carboxymethylcelluose, hydroxypropyl methylcellulose, or polyvinylpyrrolidone, emulsifying agents such as Polysorbate 80 (TWEEN-80), sequestering or chelating agents such as EDTA (ethylenediaminetetraacetic acid) or EGTA (ethylene glycol tetraacetic acid), ethyl alcohol, polyethylene glycol, propylene glycol, sodium hydroxide, hydrochloric acid, citric acid, or lactic acid. Antimicrobial agents utilized as carriers may be added to pharmaceutical compositions in multiple-dose containers that include phenols or cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride. Suitable excipients may include, for example, water, saline, dextrose, glycerol, or ethanol. Suitable non-toxic auxiliary substances may include, for example, wetting or emulsifying agents, pH buffering

agents, stabilizers, solubility enhancers, or agents such as sodium acetate, sorbitan monolaurate, triethanolamine oleate, or cyclodextrin.

[0328] The pharmaceutical compositions can be a liquid solution, suspension, emulsion, pill, capsule, tablet, sustained release formulation, or powder. Oral formulations can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, polyvinyl pyrollidone, sodium saccharine, cellulose, magnesium carbonate, etc.

[0329] In certain embodiments, the pharmaceutical compositions are formulated into an injectable composition. The injectable pharmaceutical compositions may be prepared in any conventional form, such as for example liquid solution, suspension, emulsion, or solid forms suitable for generating liquid solution, suspension, or emulsion. Preparations for injection may include sterile and/or non-pyretic solutions ready for injection, sterile dry soluble products, such as lyophilized powders, ready to be combined with a solvent just prior to use, including hypodermic tablets, sterile suspensions ready for injection, sterile dry insoluble products ready to be combined with a vehicle just prior to use, and sterile and/or non-pyretic emulsions. The solutions may be either aqueous or nonaqueous.

[0330] In certain embodiments, unit-dose parenteral preparations are packaged in an ampoule, a vial or a syringe with a needle. All preparations for parenteral administration should be sterile and not pyretic, as is known and practiced in the art.

[0331] In certain embodiments, a sterile, lyophilized powder is prepared by dissolving an antibody or antigen-binding fragment as disclosed herein in a suitable solvent. The solvent may contain an excipient which improves the stability or other pharmacological components of the powder or reconstituted solution, prepared from the powder. Excipients that may be used include, but are not limited to, water, dextrose, sorbital, fructose, corn syrup, xylitol, glycerin, glucose, sucrose or other suitable agent. The solvent may contain a buffer, such as citrate, sodium or potassium phosphate or other such buffer known to those of skill in the art at, in one embodiment, about neutral pH. Subsequent sterile filtration of the solution followed by lyophilization under standard conditions known to those of skill in the art provides a desirable formulation. In one embodiment, the resulting solution will be apportioned into vials for lyophilization. Each vial can contain a single dosage or multiple dosages of the anti-hVEGFR2 antibody or antigen-binding fragment thereof or composition thereof. Overfilling vials with a small amount above that needed for a dose or set of doses (e.g., about 10%) is acceptable so as to facilitate accurate sample withdrawal and accurate dosing. The lyophilized powder can be stored under appropriate conditions, such as at about 4° C. to room temperature.

[0332] Reconstitution of a lyophilized powder with water for injection provides a formulation for use in parenteral administration. In one embodiment, for reconstitution the sterile and/or non-pyretic water or other liquid suitable carrier is added to lyophilized powder. The precise amount depends upon the selected therapy being given, and can be empirically determined.

Methods of Use

[0333] The present disclosure also provides therapeutic methods comprising: administering a therapeutically effective amount of the antibody or antigen-binding fragment as provided herein and/or the pharmaceutical composition provided herein to a subject in need thereof, thereby treating, reducing the severity of and/or slowing the progression of a VEGFR2-related disease or condition in a subject.

[0334] In some embodiments, the VEGFR2-related disease or condition is a tumor or an angiogenic disease.

Tumors

[0335] In certain embodiments, the VEGFR2-related disease or condition is a tumor, such as a solid tumor or non-solid tumor. In some embodiments, the tumor produces VEGF (e.g., VEGF-A) and/or is sensitive to VEGF (e.g., VEGF-A) present in its microenvironment. Studies have observed VEGF production and VEGFR2 expression in solid tumors or non-solid tumors, such as human leukemias (Sato, K. et al., Tohoku *J. Exp. Med.,* 185: 173-84 (1998); Ishii, Y, Nippon Sanka Fujinka Gakkai Zasshi: 4'I: 133-40 (1995); and Ferrer, F. A. et al, Urology, 54:567-72 (1999); Fielder et al., *Blood* 89:1870-5 (1997) and Bellamy et al., *Cancer Res.* 59728-33 (1999)). Without wishing to be bound by theory, the VEGF/hVEGFR2 autocrine loop modulates tumor cell survival and migration in vivo and it has been further demonstrated that VEGFR1 monoclonal antibodies inhibited the autocrine VEGF/VEGFR1 loop in certain solid tumor cells, e.g., breast carcinoma cells, and inhibited the VEGF-stimulated migration of human leukemia cells (see, e.g., U.S. Pat. No. 7,498,414(B2)).

[0336] In certain embodiments, the solid tumor is selected from the group consisting of breast carcinoma, lung carcinoma, colorectal carcinoma, pancreatic carcinoma, glioma and lymphoma, for example, head and neck tumors, colorectal tumors, prostate tumors, breast tumors, lung tumors, such as small cell and non-small cell lung tumors, pancreatic tumors, thyroid tumors, ovarian tumors, cervical tumors, kidney tumors, brain tumors, and liver tumors, Kaposi's sarcoma, CNS neoplasms, neuroblastomas, capillary hemangioblastomas, meningiomas, cerebral metastases, melanoma, gastrointestinal and renal carcinomas and sarcomas (e.g., gastric cancer), rhabdomyosarcoma, glioblastoma, preferably glioblastoma multiforme, leiomyosarcoma, squamous cell carcinoma, basal cell carcinoma and skin cancers that can be treated by suppressing the growth of malignant keratinocytes, such as human malignant keratinocytes. In certain embodiments, the solid tumor is selected from the group consisting of gastric cancer, non-small cell lung cancer, such as large cell lung cancer.

[0337] In certain embodiments, the non-solid tumor is selected from the group consisting of leukemia, multiple myeloma and lymphoma, for example, acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), erythrocytic leukemia ormonocytic leukemia, Hodgkin's and non-Hodgkin's lymphoma.

Angiogenic Diseases

[0338] In certain embodiments, the VEGFR2-related disease or condition is an angiogenic disease. Angiogenic diseases are associated with uncontrolled angiogenesis, which can be regulated by VEGF, e.g., VEGF-A. Expression of VEGF can be found in embryonic tissues, macrophages, and proliferating epidermal keratinycytes during wound healing (Breier et al., *Development,* 114:521-32 (1992);

Brown et al., *J. Exp. Med.,* 176:1375-9 (1992); and Ferrara et al., *Endocr. Rev,* 13:18-32 (1992)). High expression of VEGF can also be found in tumors, such as glioblastoma multiforme, hemangioblastoma, central nervous system neoplasms and AIDS-associated Kaposi's sarcoma; in atherosclerotic lesions, plaques and in inflammatory cells (Nakamura, S. et al., AIDS Weekly, 13 (1) (1992); Plate, K. et al., *Nature,* 359:845-8 (1992); Plate, K. et al., *Cancer Res.,* 53:5822-7 (1993); and Berkman, R. et al., *J. Clin. Invest.,* 91:153-9 (1993)). Without wishing to be bound by theory, endothelial cells adjacent to tumor cells expressing VEGF generally show up-regulated expression of VEGF receptors, e.g., VEGFR2. Release of VEGF by tumors leads to stimulation of angiogenesis in adjacent endothelial cells, which results in proliferation, migration, differentiation, tube formation, maintenance of vascular integrity and increase in vascular permeability of the endothelial cells. Therefore, the abnormalities in the VEGF/VEGFR2 signaling involved in angiogenic diseases can be treated by the anti-VEGFR2 antibodies or antigen-binding fragments thereof provided by the present disclosure.

[0339] In some embodiments, the angiogenic disease is atherosclerosis, rheumatoid arthritis (RA), neovascular glaucoma, proliferative retinopathy including proliferative diabetic retinopathy, macular degeneration, hemangiomas, angiofibromas, psoriasis, retinopathy of prematurity (e.g., retrolental fibroplastic), corneal graft rejection, insulin-dependent diabetes mellitus, multiple sclerosis, myasthenia gravis, Chron's disease, autoimmune nephritis, primary biliary cirrhosis, acute pancreatitis, allograph rejection, allergic inflammation, contact dermatitis and delayed hypersensitivity reactions, inflammatory bowel disease, septic shock, osteoporosis, osteoarthritis, cognition defects induced by neuronal inflammation, Osler-Weber syndrome, restinosis, and fungal, parasitic and viral infections, such as cytomegaloviral infections.

[0340] In some embodiments, the subject is human.

[0341] The antibody or antigen-binding fragment as provided herein and/or the pharmaceutical composition provided herein can be administered via oral, nasal, intravenous, subcutaneous, sublingual, intratumoral, or intramuscular administration.

[0342] In some embodiments, the method provided herein further comprises administering a therapeutically effective amount of a second therapeutic agent, for example, an anti-cancer therapy, optionally the anti-cancer therapy is selected from a chemotherapeutic agent, radiation therapy, an immunotherapy agent, anti-angiogenesis agent (e.g. antagonist of a VEGFR such as VEGFR-1, VEGFR-2, and VEGFR-3), an EGFR antagonist, an PDGFR antagonist, an IGFR antagonist, an NGFR antagonist, an FGFR antagonist, a targeted therapy agent, a cellular therapy agent, a gene therapy agent, a hormonal therapy agent, cytokines, palliative care, surgery for the treatment of cancer (e.g., tumorectomy), one or more anti-emetics, treatments for complications arising from chemotherapy, or a diet supplement for cancer patients (e.g. indole-3-carbinol).

[0343] In some embodiments, the present disclosure provides kits comprising the antibody or antigen-binding fragment thereof provided herein, optionally conjugated with a detectable moiety. The kits may be useful in detection of presence or amount of hVEGFR2 in a biological sample, or may be useful in the methods of diagnosis provided herein.

[0344] In some embodiments, the present disclosure provides kits comprising the antibody or antigen-binding fragment thereof provided herein and a second therapeutic agent. The kits may be useful in treatment, prevention, and/or amelioration of VEGFR2-related disease or condition.

[0345] The present disclosure also provides a method of detecting presence or amount of VEGFR2 in a sample, comprising contacting the sample with the antibody or antigen-binding fragment thereof provided herein, and determining the presence or the amount of VEGFR2 in the sample.

[0346] In some embodiments, the present disclosure also provides use of the antibody or antigen-binding fragment thereof provided herein in the manufacture of a medicament for treating, reducing the severity of and/or slowing the progression of a VEGFR2 related disease or condition in a subject.

Examples

Example 1: Preparation and Characterization of VEGFR2 Protein

[0347] Human VEGFR2/KDR-His: Recombinant Human VEGFR/KDR Protein (hVEGFR2-his, Accession #AAI31823.1) was expressed in human 293 cells (HEK293). Briefly the coding region of the human VEGFR2 gene from Ala20-Glu764 with 6×his tag at C-terminus was used for transfection.

[0348] The supernatant was purified using His-tag affinity column. The resulting purified protein was characterized using SDS page gel. The protein was purchased from ACRO Biosystems (Cat #KDR-H5227).

[0349] Rhesus macaque VEGFR2/KDR with His tag: Recombinant Rhesus macaque VEGFR2/KDR Protein extracellular domain Ala20 to Glu764 (Accession #XP_014994176.1) was fused with polyhistidine tag at C-terminus and produced in human 293 cells (HEK293). The transfection supernatant from HEK293 cells was purified using His-tag affinity column. The resulting purified protein was characterized using SDS page gel. This protein was purchased from ACRO Biosystems (Cat #VE2-C52H3).

[0350] The above VEGFR2 proteins were used in the following experiments.

Example 2: Antibody Generation

1. Antigen Conjugation and Immunization

[0351] For immunization, the recombinant hVEGFR2-his protein was conjugated with various MabSpace immune-enhancing peptides. Briefly 2-8 fold molar excess of the peptide was mixed with Sulfo-SMCC (sulfosuccinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate, Peirce #22322)-activated hVEGFR2 protein and incubated for one hour at room temperature. The reaction was stopped and the conjugated protein was analyzed and QCed using SDS-PAGE gel.

[0352] Above conjugated hVEGFR2-his protein was emulsified in a 1:1 ratio using Complete Freund's Adjuvant (Pierce), respectively, and then immunized sub-cutaneously and intraperitoneally into C57B/L6 mice. Additional immunizations were carried out using CpG and Alum to preserve native conformation of the protein. Immunization occurred

at least every 2 weeks and anti-serum from the mice was taken after the 1st immunizations for anti-hVEGFR2 titer analysis by ELISA assay.

[0353] For determining the serum titer, 20 μl of mouse serum was prepared from each immunized mice. High-binding clear polystyrene 96 well plates (Nunc) were coated with 100 μl/well of a 1p g/ml solution consisting of human VEGFR2-his in high pH coating buffer (0.16% Na2CO3, 0.3% $NaHCO_3$, pH9.8). The plates were incubated overnight at 4° C., and then washed once on an automatic plate washer using washing buffer PBS+0.1% Tween 20 (Sigma). 200p1 of blocking buffer (PBS+1% BSA+1% Goat serum+0.05% Tween 20) was added to each well and incubated for 2 hours at room temperature. The blocking buffer was then aspirated and 100 μl of serially diluted serum in dilution buffer (PBS+1% BSA+1% Goat serum+0.01% Tween 20) was transferred to each well of the ELISA plate and allowed to incubate for 60 min at room temp. The plates were then washed 3 times using the method described above. 100p/well of solution of HRP conjugated goat anti-mouse Fc antibody (Abcam,Cat #Ab98808) diluted in dilution buffer was then added to each well of the plate. After that the ELISA plates were allowed to incubate for 60 min at RT, the plates were washed 3 times with 250 μl/well washing buffer. Finally, 100 μl/well of TMB was added to each well and the reaction was terminated using 0.64M H_2SO_4. The plates were read on a Thermo Multiscan FC at 450 nM.

2. Fusions

[0354] Four days prior to fusion, each mouse was boosted intraperitoneally with unconjugated hVEGFR2 protein in PBS. On the fusion day, the spleens were removed aseptically and the organs were processed into a single cell suspension. The red blood cells were lysed and the spleenocytes were washed with DMEM (Gibco). Viable, log-phase growth myeloma cells (SP2/0) were mixed with the murine spleenocytes in a 1:4 ratio. The cells were then washed 2 times before the fusion with PEG.

[0355] The post fusion cells were washed with DMEM and suspended in cell growth media supplemented with 10% FBS+HFCS+OPI+1X HAT. 200 μl per well of this cell suspension was plated into 96-well cell culture plates and incubated overnight in a 37° C. humidified 10% CO_2 incubator. The cultures were incubated for 7 days and then the growth media was aspirated out of the wells and exchanged for fresh growth media. Screening of hybridoma supernatants commenced 2-3 days after the media change.

3. Antibody Screening by ELISA Assay

[0356] Same protocol of determining serum titer above. Briefly, 0.5 μg/ml hVEGFR2-his was coated overnight at 4° C. After wash, 100 μl of hybridoma supernatant was added and allowed completely binding. HRP conjugated goat anti-mouse Fc antibody was then added to detect bound VEGFR2 antibody. Finally, the plates were read on a Thermo Multiscan FC at 450 nM after TMB reaction and H_2SO_4 termination. Cells from the ELISA positive hybridoma wells were subsequently expanded in cell culture for further characterization studies.

Examples 3: Subcloning of Positive Hybridoma Clones and Small Scale Antibody Production

1. Subcloning of Positive Hybridoma Clones

[0357] Cells from the ELISA positive hybridoma wells with the desired binding profile and blocking activity were selected and each plated using limited dilution in 96 well plates. These cells were allowed to grow for 7 days. Once the adequate cell mass was reached, supernatant from each well was collected and re-screened for antigen binding ability (see screening in Example 2).

[0358] From each 96 well plate, clones with highest antigen binding activity were identified and expanded with limited dilution further into 96-well plates with 2001 of hybridoma growth medium per well. After 7 days, cells from 96-well plates were tested for antigen binding. The subcloning was done more than 2 times. When more than 90 of the wells display positive binding signal, two clones with the highest antigen binding activity were identified and transferred to 24-well plates with medium and was allowed to grow for 2 additional days. Once 24 well plates were confluent, cells were transferred to 6-well plates. After 5 days of incubation, a portion of the cells were frozen down. The remainder of the cells were transferred into a flask and allowed to expand. Once the flasks were confluent, half of cells were frozen down (3 vials per clone) for additional backup. The other half was allowed to expand further in flasks with medium for antibody production. Isotypes were determined using standard methodologies.

2. Small Scale Antibody Production

[0359] Hybridoma cells were inoculated to roller bottle and cultured for 14 days with 200-300 ml of hybridoma culture medium (Invitrogen). VEGFR2 monoclonal antibodies (mAbs) were purified from hybridoma cell culture as follows. All purification processes were carried out at room temperature. One purification scheme was used to purify various mAbs and used affinity chromatography.

[0360] The host cell culture fluid (CCF) was centrifuged to remove cell debris. The CCF supernatant was then filtered, diluted and then loaded onto Protein G chromatography media in the form of a column, Protein G High Performance (Bio-Rad) and equilibrated.

[0361] After loading, the Protein G column was washed until the absorbance at 280 nm of the flow-through returned to baseline. The VEGFR2 mAb was then eluted from the column using glycine, pH 2.5 and immediately neutralized by adding 50 μL of a stock solution of 1 M Tris Base per mL of elution volume. The absorbance at 280 nm of the eluate was monitored and fractions containing protein were collected to make the Protein A pool.

[0362] Following purification, the VEGFR2 mAbs were formulated in PBS by dialysis using 10,000 MWCO membranes (Pierce Slide-A-Lyzer or dialysis tubing). Following formulation, the VEGFR2 mAbs were filtered.

Example 4: Binding Analysis of Purified VEGFR2 Antibodies

[0363] Same protocol of Example 2 antibody screening by ELISA assay. Briefly, 0.5 μg/ml hVEGFR2-his was coated and serially diluted purified antibodies would bind to coated antigen. Using HRP conjugated goat anti-mouse Fc antibody could detect binding signals of each antibody.

[0364] The data were calculated and fit by Graphpad Prism Software and EC50 of antibodies were summarized in FIG. **1**A-**1**B and Table 6. The hybridoma antibodies have similar affinity to human.

TABLE 6

EC50 values ofVEGFR2-specific antibodies obtained in ELISA binding (ng/ml)

VEGFR2-specific hybridoma antibodies	Human VEGFR2
002	16.80
003	7.69
006	13.52
018	12.95
042	22.44
048	23.20
054	8.09

Example 5: Evaluation of Blocking Activities of Purified Antibodies to Inhibit Binding of hVEGF-A to hVEGFR2

[0365] High-binding clear polystyrene 96 well plates were coated with 100 μl/well of 1 μg/ml hVEGFR2-his overnight at 4° C. After washing and blocking, serial diluted antibodies (from 10 μg/ml to 0.0006 μg/ml) were added and incubate for 1 hour at RT. 0.3 μg/ml hVEGF-A (Acrobiosystem, Cat #VE5-H4210) were added and incubated for 2 hour at RT. Then 100 μl/well of 0.25 μg/ml Biotinylated Rabbit Anti-human VEGF-A (Peprotech, Cat #400-P10Bt) was then added for detecting bound VEGF-A. Then 100 μl/well of a solution of HRP conjugated Neutravidin antibody (Pierce, Cat #31001) and TMB were added sequentially. Finally, reaction was terminated using 0.64M H_2SO_4 and plates were read at 450 nM. 1121B is a benchmark antibody (Ramucirumab) disclosed in the U.S. Pat. No. 7,498,414.

[0366] As shown in FIG. **2**, 002, 048, 054 and 1121B had similar blockade activities with comparable IC50, indicating the former 3 antibodies may also inhibit activity of VEGF-A via blocking VEGF-A/VEGFR2 interaction, like 1121B.

Example 6: Dose-Dependent Response of Binding of Purified VEGFR2 Antibodies to HUVEC Measured by FACS

[0367] Log phase HUVEC cells were collected, counted and resuspended in FACS buffer (5% BSA+PBS). 2×10/\5 cells were added to each tube and then washed once with PBS (1500 rpm, 5 mins, RT). Then 100p1/tube serial diluted VEGFR2 antibodies purified from hybridoma supernatant in FACS buffer were added into corresponding tubes and were incubated at 4° C. for 1 hr. The cells were then washed twice with 1 ml PBS, followed by adding 100 μl/tube of 2^{nd} antibody (1:400 anti-mIgG (H+L)-PE, Cell signaling #8887) in FACS buffer. The cells were incubated at 4° C. for 0.5 hr and then washed twice with PBS, followed by resuspending cells in 600 μl PBS for each sample. The cells were then transferred into FACS tube and the bindings of the antibodies to the cells were detected using flow cytometry (BD Accuri C6) (see FIG. **3**).

[0368] The percentage of positive binding cells was calculated and fit by Graphpad Prism Software. As shown in FIG. **3**, these hybridoma antibodies could bind to HUVEC cells, which is well-known of VEGFR2 expression on cell surface.

Example 7: Gene Cloning and Sequencing of Hybridoma Antibodies

[0369] The sequences of the mouse anti-human VEGFR2 antibody light chain and heavy chain variable regions were obtained by the polymerase chain reaction (PCR) amplification technique known as 5' RACE (rapid amplification of cDNA ends). Total RNA from 11B8 (002)/21B4(003)/5G4 (048)/10D11(054) antibody producing hybridoma cell was isolated using Trizol (Invitrogen) and cDNA was synthesized using Superscript first strand synthesis system (Invitrogen) with Oligo (DT) 12-18 primer (Invitrogen). The variable regions of mouse IgG gene were cloned by PCR with MuIgG VH3'-2 and MuIg VH5' leader primers for heavy chain variable region and MuIgK VL3'-1 and MuIgK VL5' leader primer for light chain variable region (NOVAGEN). The resulting band for each antibody was cloned into pMD®18-T cloning vector and DNAs from 20 clones were submitted for sequencing and determined using ABI DNA sequencing instruments (Perkin Elmer). Consensus sequences were determined using Vector NTI Advance 10 software (Invitrogen).

[0370] Generation of chimeric antibodies: After sequencing analysis and confirmation, the variable region of the each above mentioned genes were cloned into a recombinant expression vector pCP-Hck/Hcgl. Briefly, pCP-Hck/Hcgl vector was digested by two steps of restriction enzyme first. Then the genes of the light chain variable region (VL) and heavy chain variable region (VH) were homologous recombined with digested vector. After transformation, clony PCR and sequence confirmation, the expression vectors of chimeric antibodies were generated.

Example 8: Recombinant Chimeric Antibody Expression and Purification

[0371] The expression and purification of the recombinant chimeric antibody protein produced above were conducted by following methods: HEK293E cells cultured in Freestyle 293 Expression Medium with 10% of Pluronic F-68 at 1×10^6 cell/ml were transfected with DNA vector with final concentration of 0.5 μg/ml and PEI (Polyethylenimine-linear, Polyscience) of 1.0 μg/ml. DNA to PEI ratio was 1:2. DNA and PEI complexes formed period with Optimal MEM should be 15 minutes at the room temperature. Transfected cells were cultured in the flasks with 5% CO_2, at 37° C. and at 125 rpm shaking speed. 1% Peptone medium was added at 22 to 26 hours post transfection.

[0372] Conditioned medium was harvested on day 6 and supernatant was centrifuged at 3,000 rpm for 30 minutes. The clarified conditioned medium was then loaded onto a pre-equilibrated 0.5 ml Protein A column, washed with 5 column volumes of 1XPBS and finally the bound IgG was eluted with 3 mL pH3.0 0.1M Glycine-HCL buffer. The eluted antibody protein was dialyzed to PBS and stored at −80° C. To remove endotoxin, the purified protein was further processed by passing through Hitrap DEAE Sepharose F.F. column and the resulting antibody was analyzed to determine the level of purity using size exclusion chromatography (Superdex 200 5/150 GL, G.E. Healthcare).

Example 9: Binding of Purified Anti-VEGFR2 Chimeric Antibodies to Human and Rhesus VEGFR2 Protein

[0373] Same protocol of Example 4 except using HRP conjugated goat anti-human Fc antibody as secondary antibody. Binding to hVEGFR2-his and rhesus VEGFR2-his (Acrobiosystems, Cat #VE2-C52H3) were also analyzed head-to-head.

[0374] As shown in FIGS. **4**A and **4**B, 4 chimeric antibodies could bind to human and rhesus VEGFR2 with similar affinity, like 1121B, indicating the possibility of evaluating safety issue in rhesus monkey for further study.

Example 10: Characterization of the Ability of Chimeric Anti-VEGFR2 Antibodies to Inhibit the Binding VEGF-A to hVEGFR2

[0375] For this assay, 0.25 μg/ml VEGF-A was coated on ELISA plate before a mixture of chimeric antibodies and VEGFR2 were added. To detect free VEGF-A (not bind to VEGFR2), 1:1000 diluted mouse serum (mouse previous immunized with human VEGFR2) were added and HRP conjugated goat anti-mouse Fc antibody was used as secondary antibody.

[0376] As shown in FIG. **5**, 3 antibodies (042C, 048C and 054C) could inhibit VEGF-A binding to VEGFR2 as good as 1121B or even better. 002C showed much weaker neutralizing activity.

Example 11: FACS Based Analysis of Binding of Select Purified Chimeric Antibodies to HUVEC

[0377] Same protocol of Example 6 except using 1:400 rabbit anti-hIgG (H+L)-PE, (Cell signaling #8887) as secondary antibody. As shown in FIG. **6**, 002C and 054C bound to HUVEC cells with almost same EC50 of 1121B. Therefore, these VEGFR2 antibodies could function by binding to human VEGFR2 expressing on cell surface.

Examples 12: Epitope Binning by Competition Assay

[0378] Similar protocol of ELISA binding assay described above. Briefly, 0.5 μg/ml hVEGFR2-his was coated onto the plate. 20 μg/ml competitor antibodies and 1.25 μg/ml biotinylated other antibodies were added simultaneously. After incubation of 3 hours, neutravidin conjugated HRP were added for detecting bound biotinylated antibody. The signal of bound biotinylated antibody will decrease if competitor antibody compete same epitope but not effect if different epitope.

[0379] As shown in FIG. **7**, Biotin-mAb002 was competed by 002, 042, 048 and 054, indicating that they may belong to same epitope group. And this group was later found with best blocking activity and same epitope of 1121B (data not shown).

[0380] Then diluted competitor hybridoma antibodies (002, 042, 048 and 054) and lower concentration (100 ng/ml) of chimeric antibodies (002C, 042C, 054C) were added for replacement following same protocol above. Anti-hIgG Fc-HRP was used as detection antibody.

[0381] As shown in FIGS. **8**A-**8**C, with increasing concentration of hybridoma antibodies, the chimeric one could not bind well or at all. Consistent with FIGS. **7**A and **7**B, hybridoma 002, 042, 048 and 054 could compete with chimeric ones, probably due to same epitope.

Example 13: Profiling the Activities of Purified Chimeric Antibodies in Blocking VEGF-A Induced pVEGFR2 in HUVEC Cells

[0382] Cells expressing VEGFR2, such as HUVEC cell, will up regulate the expressing level of VEGFR2 upon VEGF-A stimulation. Then increased VEGFR2 could bind to VEGF-A and become dimerized form. The dimerization will induce Tyr phosphorylation of VEGFR2 following by activation of down stream pathway, such as MAPK/ERK and PI3K.

[0383] Briefly, HUVEC cells was seed into 12-well plates at a density of 1.5×10^5 cells/well in culture medium with 10% FBS and incubated for 16 hours at 37° C., 5% CO_2 incubator, then the cells were starved for 4 hours with serum free medium. Different chimeric antibodies were added to each well incubated for 30 min, followed by adding 20 or 40 ng/ml VEGF-A incubated for 15 min. Finally, cellular lysate were prepared by adding 100 μl RIPA buffer (Thermo Scientific™,Cat #89900) containing complete protease inhibitor (Roche #04693132001) and phosphatase inhibitor (Pierce #1862495), incubating on ice for 15 minutes and centrifuged to collect supernatant. Equal amounts of the cellular lysates were resolved by 8% SDS-PAGE. The proteins were transferred to a PVDF membrane (Millipore) at a tank containing ice, run 150 min under 400 amp. The membrane was then incubated with a solution containing 5% BSA+TBST for 2 hours at RT. Then the membrane was incubated with a solution containing 1% BSA+TBST and rabbit anti-phospho-VEGFR2 (Tyr1175) mAB(CST, Cat #3770)/mouse anti-β-actin antibody (Abbkine, Cat #A0101502) overnight at 4° C.; The membranes were then washed 3x with TBST for 10 min each time with gentle rotation; The membrane was then incubated for 2 hours with HRP conjugated goat anti-rabbit IgG Fc pAB(Abcam, Cat #Ab97080)/RP conjugated goat anti-mouse IgG pAb(Abcam, Cat #ab97040) 1:1000 diluted in 1% BSA+TBST. The membranes were then washed 3x with TBST for 10 min for each time with gentle rotation; Finally, 1 ml ECL mixture (Pierce) was added onto PVDF membrance for signal exposure.

[0384] As shown in FIGS. **9**A, **9**B and **9**C, both 20 and 40 ng/ml of VEGF-A could quickly induce VEGFR2 phosphorylation. And 054C, 048C, 042C and 002C could reverse this stimulation with dose-dependent manner as well as Ramucirumab.

Example 14: Generation and Characterization of Humanized Antibodies

1. Generation, Expression and Purification of Humanized Antibodies

[0385] The sequences of the variable domains of mouse antibodies 054 and 048 were used to identify the germline sequence with the highest homology to the murine framework. Computer-modelling was used to design humanized variants with CDR grafting and back mutation.

Ab-54

[0386] Human germline framework sequence VK/2D-40 for light chain and VH/3-21 for heavy chain were used for CDR grafting, respectively.

[0387] Germline sequence for Mab54 HC:

VH/3-21 (Mab54-germline, SEQ ID NO: 92):

EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISSSSSYIYYADSVKGRFTISRD

NAKNSLYLQMNSLRAEDTAVYYCARELDGNYDYWGQGTTLTVSS

-continued

VH/3-21 variant 1 (Mab54-Hzd-HC-V1, SEQ ID NO: 93):
EVQLVESGGGLVKPGGSLKLSCAASGFTFSMYGMSWVRQTPGKRLEWVASISIGGSYTYYADSVKGRFTISRD
NAKNTLYLQMNSLKAEDTAVYYCARELDGNYDYWGQGTTLTVSS

VH/3-21 variant 2 (Mab54-HC-V2, SEQ ID NO: 94):
EVQLVESGGGLVKPGGSLRLSCAASGFTFSMYGMSWVRQAPGKRLEWVASISIGGSYTYYADSVKGRFTISRD
NAKNTLYLQMNSLRAEDTAVYYCARELDGNYDYWGQGTTLTVSS

Germline sequence for Mab54 LC:
VK/2D-40 (Mab54 LC germline, SEQ ID NO: 95):
DIVMTQTPLSLPVTPGEPASISCRSSQSLLDSDDGNTYLDWYLQKPGQSPQLLIYTLSYRASGVPDRESGSGS
GTDFTLKISRVEAEDVGVYYCMQRIEFP

VK/2D-40 variant 3 (Mab54-Hzd-LC-V1, SEQ ID NO: 96):
DIVITQDELSLPVTFGESVSISCRSSKSLLYKDGKTYLNWFLQRPGQSPQLLIYLMSTRASGVSDRESGSGSG
TDFTLKISRVEAEDVGIYYCQQLVEYPFTEGSGTKLEIK

VK/2D-40 variant 4 (Mab54-Hzd-LC-V2, SEQ ID NO: 97):
DIVITQTPLSLPVTPGESVSISCRSSKSLLYKDGKTYLNWFLQRPGQSPQLLIYLMSTRASGVPDRESGSGSG
TDFTLKISRVEAEDVGVYYCQQLVEYPFTFGSGTKLEIK

Ab-48

[0388] Human germ line framework sequence VK/2D-40 for light chain and VH/3-21 for heavy chain were used for CDR grafting, respectively.

[0389] Germline sequence for Mab48 HC:

VH/3-21 (Mab48-HC germline, SEQ ID NO: 102):
EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISSSSSYIYYADSVKGRFTISRD
NAKNSLYLQMNSLRAEDTAVYYCAR

VH/3-21 variant 5 (Mab48-Hzd-HC-V3, SEQ ID NO: 98):
EVQLVESGGGLVKPGGSLRLSCAASGFTFSMYGMSWVRQAPGKRLEWVASISIGGSYTYYADSVKGRFTISRD
NAKNTLYLQMNSLRAEDTAVYYCAREMDGNYDYWGHGTTLTVSS

[0390] Germline and light chain sequence for Mab48 were same as Mab54.

[0391] Therefore, there were 4 variants of humanized Mab54, labeled as 54-H1L1, 54-H2L1, 54-H1L2, 54-H2L2 for short, and 2 variants of humanized Mab48, labeled as 48-H3L1, 48-H3L2 for short. In fact, VH/3-21 variant 5 is almost same with VH/3-21 variant 2, except one amino acid in HCDR3. That is also consistent with the similar biological activities of two antibodies shown in this patent.

[0392] The above heavy chain and light chain cDNAs were synthesized and fused with the constant region of human IgG1 in the Fc region (The numbering of the heavy chain residues described herein are according to the EU index of Kabat (see Kabat et al., "Proteins of Immunological Interest", US Dept. of Health & Human Services (1983))). The variable regions of the heavy chain and light chain of the selected antibody genes were synthesized and cloned into an expression vector and the large scale DNA was prepared using PureYield™ Plasmid Maxiprep System from Promega. Transfection was carried out using the Expi-Fectamine™ 293 Reagent from Invitrogen according to the manufacturer's protocol. Supernatants were harvested when the cell viability was around 50%. Protein A beads and clean supernatants were incubated at 4° C. for 2 hr with rocking before going through a column. The Protein A beads inside the column were washed with PBS, and 100 mM Glycine buffer (pH3.0) was used to elute the antibody, which was dialyzed against the PBS buffer (137 mM NaCl, 2.7 mM KCl 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH7.4) overnight at 4° C. Finally, endotoxin was removed using Pierce High Capacity Endotoxin Removal Resin (Invitrogen, Catalog number: 88271). The purified antibody was characterized by SDS-PAGE and SEC-HPLC.

2. Binding of Humanized Antibodies to hVEGFR2 and Rhesus VEGFR2 in ELISA

[0393] Same protocol of Example 9. Humanized 054 and 048 antibodies expressed by 293T cells were tested first (see FIGS. **10**A and **10**B).

[0394] Then two humanized antibodies 54-H2L1 and 48-H3L1 were expressed by CHO cells for further estimation, which labeled as 54-H2L1 (CHO) and 48-H3L1 (CHO). Cross-species binding to rhesus VEGFR2-his is maintained, which is a very important feature for therapeutic antibody development (see FIGS. **11**A and **11**B).

3. Specificity of Binding of Humanized VEGFR2 Antibodies

[0395] Briefly, 1 μg/ml human VEGFR1 (Sino Biological, cat #10136-H08H), VEGFR2 (Acrobiosystem, cat #KDR-H5227) and VEGFR3 (Sino Biological, cat #10806-H08H) protein were coated overnight at 4° C. 1 μg/ml humanized antibodies (48-H3L1 and 54-H2L1, produced in CHO cells or Ramucirumab from Eli Lilly Germany, lot #20150819) were added for binding to coated antigen. Secondary antibody HRP conjugated goat anti-human Fc antibody were added for detection of binding signal.

[0396] As shown in FIG. **12**, VEGFR2 antibodies 48-H3L1, 54-H2L1 and Ramucirumab only bind to VEGFR2 but not VEGFR1 or VEGFR3, indicating the high specificity of these antibodies.

4. Blocking of hVEGF-A Binding to hVEGFR2 and Rhesus VEGFR2 in ELISA

[0397] Same protocol of Example 10. Briefly, 0.25 μg/ml VEGF-A was coated overnight at 4° C. After blocking, serial diluted 54-H2L1, 48-H3L1, Ramucirumab and 1 μg/ml human VEGFR2 or rhesus VEGFR2 were added at the same time for 2 hour-incubation. After wash, mouse serum containing polyclone VEGFR2 antibody and HRP conjugated goat anti-mouse Fc antibody were added for detection of free VEGF-A coated on the plates (see FIGS. **13**A and **13**B).

[0398] As shown in FIGS. **13**A and **13**B, two humanized antibodies could block VEGF-A interacting with either human VEGFR2 or rhesus VEGFR2. The neutralizing activities of 48-H3L1 and 54-H2L1 were similar to that of Ramucirumab or may even be better.

5. Blocking of VEGF-C and VEGF-D Binding to VEGFR2 by Humanized Antibodies

[0399] Similar protocol of Example 10. Briefly, 0.5 μg/ml VEGF-A or VEGF-C or VEGF-D was coated and 30 μg/ml VEGFR2 antibodies and 2.5 μg/ml human VEGFR2 were added at the same time for 2 hour-incubation. Using same detection method of Example 10, free VEGF-A or VEGF-C or VEGF-D coated on the plates could be measured (see FIGS. **14**A, **14**B and **14**C).

[0400] As shown in FIGS. **14**A, **14**B and **14**C, VEGFR2 antibodies, including 1121B, completely blocked VEGF-A binding to VEGFR2 but not VEGF-C or VEGF-D. Although a little decrease of VEGF-C binding was seen, the window was too limited to draw a conclusion.

6. Bindings of Humanized Antibodies to hVEGFR2 on HUVEC by FACS

[0401] Same methods with Example 11. Briefly, Log phase HUVEC cells were collected and added to each tube. Then 100 μl/tube serial diluted humanized antibodies in FACS buffer were added and kept at 4° C. for 1 hr. Binding of antibodies to HUVEC was detected by PE signal of the 2^{nd} antibody (anti-hIgG (H+L)-PE), which was added after the 1^{st} antibodies. (see FIG. **15**).

7. Blocking of hVEGF-A Induced hVEGFR2 Phosphorylation in HUVEC

[0402] Same methods of Example 13. Briefly, HUVEC cells was seed into 12-well plates in culture medium with 10% FBS and incubated for 16 hours, then starved for 4 hours without serum. Diluted humanized antibodies were added to each well incubated for 30 min, followed by adding 40 ng/ml VEGF-A incubated for 15 min. Finally, the cellular lysate were prepared and detected phospho-VEGFR2 and j-actin by Western Blot described in Example 13. (see FIG. **16**).

[0403] As shown in FIG. **16**, two variants of humanized 54 could decrease the amount of phosphorylated VEGFR2 induced by VEGF-A. The inhibition effect of 54-H2L1 was more potent than 54-H1L1.

Example 15: Evaluation of the Ability of the Purified Humanized Antibodies to Inhibit HUVEC Proliferation and Tube Formation

[0404] Log-phased HUVEC cells were seed into 96-well plates at a density of 5×10^3 cells/well in culture medium with 10% FBS and incubated overnight at 37° C., 5% CO_2 incubator, then the cells were starved for 4 hours with 50 μl/well of serum free medium. Serial diluted humanized antibodies were added 50 μl/well and incubated for 30 min, followed by adding 50p/well VEGF-A (final concentration: 20 ng/ml) and incubated for 72 hours. At the end of cell culture, 20p1/well of Cell Titer-Glo® Luminescent kit was added and the supernatant was transferred to a new white plate for luminescent signal reading.

[0405] HUVEC cells stimulated by VEGF family will be activated and proliferate. Blockade of VEGFR2 and VEGF could reverse the stimulation effect. As shown in FIG. **17**, humanized 54 and 48 antibodies could inhibit proliferation as well as Ramucirumab with dose-dependent manner, indicating that they have blocked VEGF/VEGFR2 signal pathway of HUVEC cells.

Example 16: Evaluation of the Binding Affinity of Select Mouse Hybridoma or Humanized Anti-VEGFR2 Antibodies Via Biacore

[0406] A CM5 sensor chip was activated in each flow cell by 7-min injection (10 μl/min) of freshly prepared 1:1 50 mM N-hydroxysuccinimide (NHS): 200 mM 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC). Then anti-human Fc antibody (GE Healthcare) in a concentration of 10 μg/ml in 10 mM sodium acetate buffer PH 5.0 was injected onto the activated chip at 10 μl/min (HBS-EP running buffer: 10 mM HEPES, 150 mM NaCl, 3.4 mM EDTA, 0.005% surfactant P20, pH 7.4). The remaining active coupling sites were blocked by 7 min injection of 1M ethanolamine at 10 μl/min. The immobilization level of each flow cell is ~9000RU. Antibodies were captured in FC2 by anti-human Fc IgG or anti-mouse Fc (GE Healthcare) to 200~300RU. FC1 was used as the reference cell. Antigen was injected at varying concentrations (2.5 nM, 5 nM, 10 nM, 20 nM, 40 nM and 80 nM) after capture of antibody. The association time for antibody binding antigen is 180s. Surface Regeneration condition is 120s at 10 μl/min in Gly pH1.5. The signals with captured antibody subtracted from those without captured antibody were calculated with Biacore X100 evaluation software ver. 2.0 (Biacore).

TABLE 7

Affinity parameters of antibodies binding to antigen

Antibody	clones	ka (1/Ms)	kd (1/s)	KD (M)
mAb002	11B8C2C11	5.62E+04	3.36E−04	5.98E−09
mAb003	21B4G9B4	1.26E+05	2.95E−05	2.35E−10
mAb006	27E8A9D5	1.27E+05	1.86E−04	1.47E−09
mAb018	16D9G2E8	8.84E+04	4.52E−04	5.11E−09
mAb042	8G11G1B2C6	8.51E+04	4.88E−05	5.73E−10
mAb054	10D11F4D1F6	9.18E+04	9.12E−05	9.93E−10
mAb048	5G4H2C3D3	1.14E+06	<1.0E−7	<1.0E−12
mAb054	10D11F4D1F6	9.70E+04	1.22E+06	1.26E−10
mAb054	54-H1L1	4.51E+04	5.15E−05	1.14E−09
mAb054	54-H1L2	3.77E+04	1.39E−04	3.68E−09
mAb054	54-H2L1	5.48E+04	1.38E−04	2.51E−09
mAb054	54-H2L2	2.97E+04	1.28E−04	4.30E−09
mAb054	54-C	4.60E+04	1.13E−04	2.45E−09

[0407] It is known that the interaction between human VEGF-A and VEGFR2-Fc has a K_D of about 89 pM (doi: 10.1007/s10456-011-9249-6).

Example 17: Epitope Mapping of Select Anti-VEGFR2 Via Alanine Scan

1. Generation of Mutant Human VEGFR2 Recombinant Proteins

[0408] The cDNA coding for extracellular human VEGFR2 (amino acid 1-1356) and Fc fragment of human

IgG1 was synthesized in vitro (SEQ ID NO: 103 is the amino acid sequence). Variants of human VEGFR2 with single amino acid change at designated position as listed below were amplified by overlapping PCR as described below and using primers (Dr. Oligo BLP-192, Biolytic) as shown in (Table 8). The resulting fragment was digested with restriction enzymes for Hind III and BamH I at 5' and 3' ends respectively. The PCR product was then cloned into the pcDNA3.1 (+) vector by method of homologous recombination using Syno assembly mix reagent (Synbio) following manufacturer's instructions. Plasmid was purified QIAGEN Plasmid Mega Kit (QIAGEN).

TABLE 8-continued

Variants mutant primers sequence
Initial denaturation: 98° C. 1 min
denaturation: 98° C. 15 s
Annealing: 58° C. 30 s
Extension: 72° C. 30 s per Kb
Eventually extension: 72° C. 3 mins 30 cycles

Amino acid sequence (SEQ ID NO: 103):
MQSKVLLAVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTIKANTTLQITCRGQRDLDWLWPNNQ

SGSEQRVEVTECSDGLFCKTLTIPKVIGNDTGAYKCFYRETDLASVIYVYVQDYRSPFIASVSDQHG

VVYITENKNKTVVIPCLGSISNLNVSLCARYPEKRFVPDGNRISWDSKKGFTIPSYMISYAGMVFCE

AKINDESYQSIMYIVVVVGYRIYDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDENWEYPSSKHQ

HKKLVNRDLKTQSGSEMKKELSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKPFVAFGSG

MESLVEATVGERVRIPAKYLGYPPPEIKWYKNGIPLESNHTIKAGHVLTIMEVSERDTGNYTVILTN

PISKEKQSHVVSLVVYVPPQIGEKSLISPVDSYQYGTTQTLTCTVYAIPPPHHIHWYWQLEEECANE

PSQAVSVINPYPCEEWRSVEDFQGGNKIEVNKNQFALIEGKNKTVSTLVIQAANVSALYKCEAVNKV

GRGERVISFHVTRGPEITLQPDMQPTEQESVSLWCTADRSTFENLTWYKLGPQPLPIHVGELPTPVC

KNLDTLWKLNATMFSNSINDILIMELKNASLQDQGDYVCLAQDRKTKKRHCVVRQLTVLERVAPTIT

GNLENQTTSIGESIEVSCTASGNPPPQIMWFKDNETLVEDSE

[0409] Using wild-type VEGFR2 plasmid generated above (SEQ ID NOs: 103 and 104) as template, two segments of an integrated sequence were generated with mega primers (Table 8), and ligation was accomplished by homologous recombination. The product was then cloned into the pcDNA3.1 (+) vector, after screening the individual positive colony through sequencing to identify the variants, VEGFR2 mutants had been proved to be generated successfully. PCR procedure and conditions as follows:

TABLE 8

Variants mutant primers sequence	
Step 1: to generate two mega fragments of variants	
	μl
ddH2O	35
5 × S15 PCR buffer	10
10 mM dNTP	1
F primer	1
R primer	1
PCR product	1
S15 polymerase	1
	50

TABLE 8-continued

Variants mutant primers sequence	
Step 2: to join the two pieces together Set up the following reaction on ice (homologous recombination):	
Syno assembly mix	10 μl
Sequence products 1	2 μl
Sequence products 2	2 μl
Deionized H2O	6 μl
Total Volume	20 μl

[0410] Other 20 variants are constructed by another company, Genewiz (Suzhou, China). Similar methods with Synbio Tech are performed to synthesize VEGFR2 variants.

[0411] Subsequently, these plasmids of mutants and wild-type VEGFR2 were transfected into 293T (ATCC® CRL3216) cell line. First, Seeding 5×10^6 293T cells into 60 mm dish, make sure primary ratio is at 60%~80% for transfection. Then dilute 10 g DNA in 400 μl 1×HBS, incubate for about 5 min. Add 10 125 kDa linear PEI transfection reagent (dissolved in 1×HBS, 1 mg/ml stock solution) to the above mixture, make sure DNA/PEI ratio is 1:2.5. Then the mixture was added into 293T dish drop by drop. Change the medium and replace with complete DMEM about 6-8 hrs later. After 72 hrs, cell culture supernatant was collected with 0.22 m filter respectively, then stored at −80° C. for use.

2. Binding of VEGFR2 Antibodies to hVEGFR2 Mutants by ELISA

[0412] Supernatant was used to detect the binding of VEGFR2 antibodies by ELISA as described below.

2.1 for Mouse Abs:

[0413] 0.5 μg/ml anti-human Fe antibody (Abcam) was coated for one hour at room temperature. 160 μl DMEM supernatant containing various mutant human VEGFR2-Fc proteins or 500 ng/ml wild type human VEGFR2-Fc protein in DMEM were added to each well and incubated for 1 hour at room temperature. Mouse anti-VEGFR2 antibodies at 0.5 μg/ml were added and incubated for 1 hour at RT. Then HRP conjugated goat anti-mouse IgG Fe antibody and TMB were added sequentially and the reaction was terminated using 0.64M H_2SO_4. The plates were read on a Thermo Multiscan FC at 450 nM.

2.2 for Abs with Human Fc (Chimeric or Humanized Antibodies):

[0414] Similar protocol of 2.1 except adding chimeric or humanized antibodies for binding to mutant VEGFR2-Fc and HRP conjugated anti-hIgG Fe as secondary antibody.

[0415] Table 9 summarizes the key residues on hVEGFR2 that are required for each individual antibody tested to human VEGFR2 in the ELISA assay. The amino acid residue mutations on the human VEGFR2 proteins that lead to significantly reduced binding signal relative to that of the wild type protein is marked.

TABLE 9

Summary of the key residues on hVEGFR2 for each antibody

Code	Position	Mutant	02	02C	42	42C	48	48C	54	54C	1121b
1		KDR WT									
8	133	H133A									
21	135	V135A									
9	137	Y137A		★★							
22	164	R164A			★				★		
10	165	Y165A	★		★	★	★	★	★	★	★
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11	215	I215A									
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12	218	V218A		★★							
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25	253	N253A		★★★				★		★★	
16	255	G255A		★★★		★★		★★		★★	★
4	257	D257A		★★	★★★	★★★		★★★	★★	★★★	★★★
17	259	N259A									
5	261	E261A									
14	286	K286A		★★							
18	312	G312A				★★		★★		★★	
6	313	L313A	★★★	★★★		★★		★★★		★★	★★
19	314	M314A		★		★★		★★		★★	
7	315	T315A		★★★		★★★		★★★		★★★	★★
20	316	K316A		★		★★		★★		★★	

Example 18: Large Scale Production of Anti-VEGFR2 Antibodies for In Vivo Studies

[0416] Antibodies were produced using transient expression in CHO-K1 cells. The produced antibodies were purified using protein-A affinity column and upon desalting, the antibodies were formulated into PBS at 5 mg/ml with endotoxin level at <3 unit/mg. The resulting antibodies were characterized for purity using SDS-PAGE gel and SEC-HPLC.

Transient Expression and Purification of Recombinant Antibody Using CHO-K1 Cells

[0417] The variable region of the heavy chain and light chain selected antibody genes were synthesized and cloned into an expression vector. The resulting expression contrast was used to transfect CHO-K1 cell adapted to grow in serum free medium. After 10 days' growth in 10 L Applikon bioreactor, the culture medium was harvested and the cells and cell debris were removed using ultra-filters. The clarified supernatant was then concentrated by ultrafiltration and uploaded onto a prepared Protein A (human IgG) or G-sepharose (mouse IgG) column. After being washed with equilibration buffer to baseline under monitoring by a UV monitor, the column was then eluted with 0.1 M citric acid, pH 3.5, and the eluted antibody was immediately neutralized with 1.0 M Tris-HCl buffer, pH 8.0, and dialyzed against PBS, pH 7.2 (Invitrogen), overnight at 2-8° C. with 2 buffer exchanges. The purified antibody was filtered through 0.22 μm sterile syringe filters and stored in aliquots at −80° C. or below.

Example 19: In Vivo Evaluation of the Chimeric Anti-Human VEGFR2 Antibodies in HL-60 Tumor Model

[0418] In order to evaluate anti-tumor efficacy of VEGFR2 candidate antibodies, HL-60 mouse model was set up and many symptoms were estimated for efficacy. Briefly, 4-6 weeks female NOD/SCID mice were randomized grouped into 4 groups. After treatment of CTX (150mpk, i.p.), 1.5×10^7 cells/mice were injected by i.v the next day. And 3 days after HL-60 cells injection, mice were treated with different antibodies listed in following table (Table 10).

TABLE 10

Grouping and schedule of treatment (n = 6)

Group	Dosage	Schedule of treatment
Negative Control (saline)	—	twice/week × 4
1121B	5 mpk	twice/week × 4
54-C	5 mpk	twice/week × 4
2-C	5 mpk	twice/week × 4

[0419] As shown in FIG. **18** and Table 11, 54-C had best survival percentage and condition, comparing with other groups. No animal died with 54-C treatment but 2 died in negative control group and 2-C treatment group. As we know, VEGFR2 activation occurs via binding to its ligand VEGF-A that mediates HL-60 cell proliferation through autocrine signaling. This may explain the tumor inhibition of VEGFR2 neutralizing antibody 54-C and 1121B.

TABLE 11

Symptoms and death of each treatment groups

Group	Hindlimb Weakness	Hair Disheveled	Swollen Head	Thin	Death
Negative Control	4/4	3/4	4/4	2/4	2/6
1121B	4/5	4/5	4/5	0/6	1/6
54-C	5/6	3/6	5/6	2/6	0/6
2-C	3/4	3/4	3/4	0/4	2/6

Example 20: Evaluation of the Anti-Mouse VEGFR2 Antibody DC101

1. In Vitro Evaluation of the Anti-Mouse VEGFR2 Antibody DC101

[0420] We recombined and expressed anti-mouse VEGFR2 antibody DC101 according to its sequence of Fab region listed in the Table 12 below. For Fc region of DC101, rat IgG1 Fc was replaced with human IgG1 Fc and named as chimeric-DC101. Then chimeric-DC101 was evaluated by in vitro assays to confirm its bioactivity.

TABLE 12

Variable chain sequences of DC101

	VH	VL
DC101	SEQ ID NO: 40 **MEFGLSWLFLVAILKGVQC**EVQLVETGGGLVQPGNSLKLSCAT SGFIFSTTWMNWIRQTPGKRLEWLAQIEDKSNNYFISYSESVKG RFTISRDDSKSSVYLQMNNLKEEDTAIYYCSWKYRSNYYFDYWG QGVMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	SEQ ID NO: 99 **MDMRVPAQLLGLLLLWFPGSRC**DIVLTQSP ALAVSLEQRATISCKTSQNVDYYGISYLHWYQ QKPGQQPKLLIYEGSNLASGIPARFSGSGSGT DFTLTIDPVEADDIVTYYCQQSKDYPYTFGAG TKLELKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC

Sequences in bold in the Table 12 represent the signal peptide of the VH and VL respectively.

[0421] ELISA binding assay was conducted to confirm its binding affinity. Briefly, ELISA plates were coated with 500 ng/mL mouse VEGFR2 in coating buffer (0.16% Na_2CO_3, 0.30% $NaHCO_3$) and incubated at 4° C. overnight. After twice washing, blocking buffer (1% BSA, 1% goat serum, 0.05% Tween20 in PBS) was added and plates were incubated at room temperature for 2 hours. After three times washing, serial diluted chimeric-DC101 was added in dilution buffer (1% BSA, 1% goat serum, 0.01% Tween20 in PBS) and plates were incubated at room temperature for 3 hours. After three times washing, goat anti-human HRP (1:5000 diluted) was added and plates were incubated at room temperature for 1 hour. Finally, after three times washing, TMB was added and 4 mins later 0.16 mol/L sulfuric acid was added to terminate the reaction. OD450 nm was read and recorded.

[0422] As shown in FIG. **19**, three lots of chimeric-DC101 had very consistent binding curve and EC_{50}, around 0.06 μg/mL, which was also quite similar to EC_{50} of humanized 54 antibodies and 48 antibodies.

[0423] ELISA blocking assay was conducted to confirm its VEGF blockade activity. Briefly, ELISA plates were coated with 250 ng/mL recombinant human VEGF165 in coating buffer and incubated at 4° C. overnight. After twice washing, blocking buffer was added and plates were incubated at room temperature for 2 hours. After three times washing, serial diluted chimeric-DC101 was added in dilution buffer and then 1000 ng/mL mouse VEGFR2 was added. Plates were incubated at room temperature for 3 hours. After three times washing, polyclonal anti-mouse VEGFR2 with mouse Fc (1: 1000) was added and plates were incubated at room temperature for 1 hour. After three times washing, goat anti-mouse HRP (1:5000 diluted) was added and plates were incubated at room temperature for 1 hour. Finally, after three times washing, TMB was added and 4 mins later 0.16 mol/L sulfuric acid was added to terminate the reaction. OD450 nm was read and recorded.

[0424] As shown in FIG. **20**, three lots of chimeric-DC101 had consistent blocking curves and IC_{50} values, which were quite similar to those of humanized anti-human VEGFR2 antibodies 054 and 048. Chimeric-DC101 was shown to have similar binding affinity and blocking activity with those of humanized 054 and 048 antibodies, indicating it can be used as surrogated antibody of 054 and 048 antibodies.

2. In Vivo Evaluation of the Anti-Mouse VEGFR2 Antibody DC101 Using MKN45 Gastric Tumor Model

[0425] MKN45 cells were maintained in vitro as a monolayer culture in RPMI1640 medium (Thermo Fisher) supple-

mented with 10% heat inactivated fetal bovine serum (Ex-Cell Biology), 100 U/ml penicillin and 100 ug/ml streptomycin (Hyclone) at 37° C. in an atmosphere with 5% CO_2 in air. The tumor cells were routinely sub-culture twice weekly by trypsin-EDTA treatment (Hyclone). The cells growing in an exponential growth phase were harvested and counted for tumor inoculation. Female SPF grade nude mice were inoculated with mixed 5*10/\6 MKN45 cells with 50% matrigel. When the tumor size around 100 mm/\3, tumor bearing mice were selected and randomized to 5 groups (n=10). Animals were treated with 30 mg/kg isotype control, 3 mg/kg chimeric-DC101, 10 mg/kg chimeric-DC101, 30 mg/kg chimeric-DC101 and 5 mg/kg Docetaxel as positive control, isotype control and chimeric-DC101 were administrated twice a week for 3 weeks by i.p. injection, while Docetaxel was administrated once a week for 3 weeks by i.v. injection. Tumor size was measured twice a week in two dimensions using a caliper (INSIZE) and the volume was expressed in mm/\3 using the formula: V=0.5 a*b/\2 where a and b ate the long and shirt diameters of the tumor, respectively. Results were analyzed using Prism GraphPad and expressed as mean±S.E.M. Comparisons between two groups were made by T-test, and the difference is considered significant if p is *<0.05 and **<0.01.

[0426] As shown in Table 12 and FIG. **21**, 3 mg/kg chimeric-DC101 has significantly inhibited MKN45 tumor growth, compared with the isotype control group. With the increase of dose, the tumor inhibition rate also increased, from 54% to 77%, indicating the tumor growth inhibition was induced by chimeric-DC101 and dose-dependent.

TABLE 12

Tumor Growth Inhibition of chimeric-DC101 on MKN45 xenograft tumor model on Day 22

Treatment (n = 10)	Tumor size (mm^3, mean ± S.E.M.)	TGI (%)	p value vs Isotype control
30 mg/kg isotype control	678.65 ± 20.35	/	/
3 mg/kg chimeric-DC101	242.70 ± 23.42	53.76	3.8*10^ − 11
10 mg/kg chimeric-DC101	206.69 ± 20.18	63.96	2.7*10^ − 12
30 mg/kg chimeric-DC101	109.35 ± 13.11	76.85	5.8*10^ − 15
5 mg/kg Docetaxel	190.07 ± 25.21	69.98	1.2*10^ − 11

[0427] 3. In Vivo Evaluation of the Anti-Mouse VEGFR2 Antibody DC101 Using H460 Lung Tumor Model

[0428] H460 cells were maintained in vitro as a monolayer culture in RPMI1640 medium (Thermo Fisher) supplemented with 10% heat inactivated fetal bovine serum (Ex-Cell Biology), 100 U/ml penicillin and 100 ug/ml streptomycin (Hyclone) at 37° C. in an atmosphere with 5% CO2 in air. The tumor cells were routinely sub-culture twice weekly by trypsin-EDTA treatment (Hyclone). The cells growing in an exponential growth phase were harvested and counted for tumor inoculation. Female SPF grade nude mice were inoculated with mixed 5*10/\6 H460 cells with 50% matri-gel. When the tumor size around 150 mm/\3, tumor bearing mice were selected and randomized to 5 groups (n=10). Animals were treated with 30 mg/kg isotype control, 3 mg/kg chimeric-DC101, 10 mg/kg chimeric-DC101, 30 mg/kg chimeric-DC101 and 5 mg/kg Docetaxel as positive control, isotype control and chimeric-DC101 were administrated twice a week for 2 weeks by i.p. injection, while Docetaxel was administrated once in 5 days for 3 doses by i.v. injection. Tumor size was measured twice a week in two dimensions using a caliper (INSIZE) and the volume was expressed in mm/\3 using the formula: V=0.5 a*b/\2 where a and b ate the long and shirt diameters of the tumor, respectively. Results were analyzed using Prism GraphPad and expressed as mean±S.E.M. Comparisons between two groups were made by T-test, and the difference is considered significant if p is *<0.05 and **<0.01.

[0429] As shown in Table 13 and FIG. **22**, 3 mg/kg chimeric-DC101, as the lowest dose, seemed to be not enough for inhibiting H460 tumor growth. When the dose reached 10 mg/kg and 30 mg/kg, H460 growth was significantly affected. The dose-dependent efficacy was obvious, indicating it was caused by chimeric-DC101.

TABLE 13

Tumor Growth Inhibition of chimeric-DC101 on H460 xenograft tumor model on Day 15

Treatment (n = 10)	Tumor size (mm^3, mean ± S.E.M.)	TGI (%)	p value vs Isotype control
30 mg/kg isotype control	1463.91 ± 118.72	/	/
3 mg/kg chimeric-DC101	1212.70 ± 136.42	17.16	0.1817
10 mg/kg chimeric-DC101	845.98 ± 77.85	42.21	0.0004
30 mg/kg chimeric-DC101	541.96 ± 61.33	62.98	1.9*10^ − 6
5 mg/kg Docetaxel	765.28 ± 120.64	47.72	0.0006

4. In Vivo Evaluation of the Anti-Mouse VEGFR2 Antibody DC101 Using H1975 NSCLC Tumor Model

[0430] H1975 cells were maintained in vitro as a monolayer culture in RPMI1640 medium (Thermo Fisher) supplemented with 10% heat inactivated fetal bovine serum (Ex-Cell Biology), 100 U/ml penicillin and 100 ug/ml streptomycin (Hyclone) at 37° C. in an atmosphere with 5% CO_2 in air. The tumor cells were routinely sub-culture twice weekly by trypsin-EDTA treatment (Hyclone). The cells growing in an exponential growth phase were harvested and counted for tumor inoculation. Female SPF grade nude mice were inoculated with mixed 5*10/\6 H1975 cells with 50% matri-gel. When the tumor size around 100 mm/\3, tumor bearing mice were selected and randomized to 5 groups (n=10). Animals were treated with 30 mg/kg isotype control, 3 mg/kg chimeric-DC101, 10 mg/kg chimeric-DC101, 30 mg/kg chimeric-DC101 and 5 mg/kg Docetaxel as positive control, isotype control and chimeric-DC101 were administrated twice a week for 2 weeks by i.p. injection, while Docetaxel was administrated once a week for 2 weeks by i.v. injection. Tumor size was measured twice a week in two dimensions using a caliper (INSIZE) and the volume was expressed in mm/\3 using the formula: V=0.5 a*b/\2 where a and b ate the long and shirt diameters of the tumor, respectively. Results were analyzed using Prism GraphPad and expressed as mean±S.E.M. Comparisons between two groups were made by T-test, and the difference is considered significant if p is *<0.05 and **<0.01.

[0431] As shown in Table 14 and FIG. **23**, chimeric-DC101 has significant tumor inhibition effect on H1975 xenograft tumor model, which belongs to NSCLC adenocarcinoma. With the increase of dose, from 3 mg/kg to 30 mg/kg, the tumor inhibition was more and more significant, from 40% to 80%. The dose-dependent effect indicated that the inhibition was caused by chimeric-DC101.

TABLE 14

Tumor Growth Inhibition of chimeric-DC101 on H1975 xenograft tumor model on Day 15

Treatment (n = 10)	Tumor size (mm^3, mean ± S.E.M.)	TGI (%)	p value vs Isotype control
30 mg/kg isotype control	1237.37 ± 54.49		
3 mg/kg chimeric-DC101	745.90 ± 102.69	39.72	5.1*10^ − 3
10 mg/kg chimeric-DC101	470.00 ± 69.44	62.02	7.4*10^ − 8

TABLE 14-continued

Tumor Growth Inhibition of chimeric-DC101 on H1975 xenograft tumor model on Day 15

Treatment (n = 10)	Tumor size (mm^3, mean ± S.E.M.)	TGI (%)	p value vs Isotype control
30 mg/kg chimeric-DC101	253.72 ± 33.94	79.50	9.0*10^ − 12
5 mg/kg Docetaxel	330.54 ± 78.70	73.29	2.0*10^ − 8

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65                  70                  75                  80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            100                 105                 110

Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                165                 170                 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            180                 185                 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        195                 200                 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    210                 215                 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
225                 230                 235                 240

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                245                 250                 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            260                 265                 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        275                 280                 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
    290                 295                 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305                 310                 315                 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                325                 330


<210> SEQ ID NO 39
<211> LENGTH: 1356
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 39

Met Gln Ser Lys Val Leu Leu Ala Val Ala Leu Trp Leu Cys Val Glu
1               5                   10                  15

Thr Arg Ala Ala Ser Val Gly Leu Pro Ser Val Ser Leu Asp Leu Pro
            20                  25                  30

Arg Leu Ser Ile Gln Lys Asp Ile Leu Thr Ile Lys Ala Asn Thr Thr
        35                  40                  45

Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu Asp Trp Leu Trp Pro
    50                  55                  60
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Asn Asn Gln Ser Gly Ser Glu Gln Arg Val Glu Val Thr Glu Cys Ser
65                  70                  75                  80

Asp Gly Leu Phe Cys Lys Thr Leu Thr Ile Pro Lys Val Ile Gly Asn
                85                  90                  95

Asp Thr Gly Ala Tyr Lys Cys Phe Tyr Arg Glu Thr Asp Leu Ala Ser
            100                 105                 110

Val Ile Tyr Val Tyr Val Gln Asp Tyr Arg Ser Pro Phe Ile Ala Ser
        115                 120                 125

Val Ser Asp Gln His Gly Val Val Tyr Ile Thr Glu Asn Lys Asn Lys
    130                 135                 140

Thr Val Val Ile Pro Cys Leu Gly Ser Ile Ser Asn Leu Asn Val Ser
145                 150                 155                 160

Leu Cys Ala Arg Tyr Pro Glu Lys Arg Phe Val Pro Asp Gly Asn Arg
                165                 170                 175

Ile Ser Trp Asp Ser Lys Lys Gly Phe Thr Ile Pro Ser Tyr Met Ile
            180                 185                 190

Ser Tyr Ala Gly Met Val Phe Cys Glu Ala Lys Ile Asn Asp Glu Ser
        195                 200                 205

Tyr Gln Ser Ile Met Tyr Ile Val Val Val Val Gly Tyr Arg Ile Tyr
    210                 215                 220

Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu
225                 230                 235                 240

Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile
                245                 250                 255

Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu
            260                 265                 270

Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe
        275                 280                 285

Leu Ser Thr Leu Thr Ile Asp Gly Ile Thr Arg Ser Asp Gln Gly Leu
    290                 295                 300

Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr
305                 310                 315                 320

Phe Val Arg Val His Glu Lys Pro Phe Val Ala Phe Gly Ser Gly Met
                325                 330                 335

Glu Ser Leu Val Glu Ala Thr Val Gly Glu Arg Val Arg Ile Pro Ala
            340                 345                 350

Lys Tyr Leu Gly Tyr Pro Pro Pro Glu Ile Lys Trp Tyr Lys Asn Gly
        355                 360                 365

Ile Pro Leu Glu Ser Asn His Thr Ile Lys Ala Gly His Val Leu Thr
    370                 375                 380

Ile Met Glu Val Ser Glu Arg Asp Thr Gly Asn Tyr Thr Val Ile Leu
385                 390                 395                 400

Thr Asn Pro Ile Ser Lys Glu Lys Gln Ser His Val Val Ser Leu Val
                405                 410                 415

Val Tyr Val Pro Pro Gln Ile Gly Glu Lys Ser Leu Ile Ser Pro Val
            420                 425                 430

Asp Ser Tyr Gln Tyr Gly Thr Thr Gln Thr Leu Thr Cys Thr Val Tyr
        435                 440                 445

Ala Ile Pro Pro Pro His His Ile His Trp Tyr Trp Gln Leu Glu Glu
    450                 455                 460
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Glu Cys Ala Asn Glu Pro Ser His Ala Val Ser Val Thr Asn Pro Tyr
465                 470                 475                 480

Pro Cys Glu Glu Trp Arg Ser Val Glu Asp Phe Gln Gly Gly Asn Lys
                485                 490                 495

Ile Glu Val Asn Lys Asn Gln Phe Ala Leu Ile Glu Gly Lys Asn Lys
            500                 505                 510

Thr Val Ser Thr Leu Val Ile Gln Ala Ala Asn Val Ser Ala Leu Tyr
        515                 520                 525

Lys Cys Glu Ala Val Asn Lys Val Gly Arg Gly Glu Arg Val Ile Ser
    530                 535                 540

Phe His Val Thr Arg Gly Pro Glu Ile Thr Leu Gln Pro Asp Met Gln
545                 550                 555                 560

Pro Thr Glu Gln Glu Ser Val Ser Leu Trp Cys Thr Ala Asp Arg Ser
                565                 570                 575

Thr Phe Glu Asn Leu Thr Trp Tyr Lys Leu Gly Pro Gln Pro Leu Pro
            580                 585                 590

Ile His Val Gly Glu Leu Pro Thr Pro Val Cys Lys Asn Leu Asp Thr
        595                 600                 605

Leu Trp Lys Leu Asn Ala Thr Met Phe Ser Asn Ser Thr Asn Asp Ile
    610                 615                 620

Leu Ile Met Glu Leu Lys Asn Ala Ser Leu Gln Asp Gln Gly Asp Tyr
625                 630                 635                 640

Val Cys Leu Ala Gln Asp Arg Lys Thr Lys Lys Arg His Cys Val Val
                645                 650                 655

Arg Gln Leu Thr Val Leu Glu Arg Val Ala Pro Thr Ile Thr Gly Asn
            660                 665                 670

Leu Glu Asn Gln Thr Thr Ser Ile Gly Glu Ser Ile Glu Val Ser Cys
        675                 680                 685

Thr Ala Ser Gly Asn Pro Pro Pro Gln Ile Met Trp Phe Lys Asp Asn
    690                 695                 700

Glu Thr Leu Val Glu Asp Ser Gly Ile Val Leu Lys Asp Gly Asn Arg
705                 710                 715                 720

Asn Leu Thr Ile Arg Arg Val Arg Lys Glu Asp Glu Gly Leu Tyr Thr
                725                 730                 735

Cys Gln Ala Cys Ser Val Leu Gly Cys Ala Lys Val Glu Ala Phe Phe
            740                 745                 750

Ile Ile Glu Gly Ala Gln Glu Lys Thr Asn Leu Glu Ile Ile Ile Leu
        755                 760                 765

Val Gly Thr Ala Val Ile Ala Met Phe Phe Trp Leu Leu Leu Val Ile
    770                 775                 780

Ile Leu Arg Thr Val Lys Arg Ala Asn Gly Gly Glu Leu Lys Thr Gly
785                 790                 795                 800

Tyr Leu Ser Ile Val Met Asp Pro Asp Glu Leu Pro Leu Asp Glu His
                805                 810                 815

Cys Glu Arg Leu Pro Tyr Asp Ala Ser Lys Trp Glu Phe Pro Arg Asp
            820                 825                 830

Arg Leu Lys Leu Gly Lys Pro Leu Gly Arg Gly Ala Phe Gly Gln Val
        835                 840                 845

Ile Glu Ala Asp Ala Phe Gly Ile Asp Lys Thr Ala Thr Cys Arg Thr
    850                 855                 860

Val Ala Val Lys Met Leu Lys Glu Gly Ala Thr His Ser Glu His Arg
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865                 870                 875                 880

Ala Leu Met Ser Glu Leu Lys Ile Leu Ile His Ile Gly His His Leu
                885                 890                 895

Asn Val Val Asn Leu Leu Gly Ala Cys Thr Lys Pro Gly Gly Pro Leu
            900                 905                 910

Met Val Ile Val Glu Phe Cys Lys Phe Gly Asn Leu Ser Thr Tyr Leu
        915                 920                 925

Arg Ser Lys Arg Asn Glu Phe Val Pro Tyr Lys Thr Lys Gly Ala Arg
    930                 935                 940

Phe Arg Gln Gly Lys Asp Tyr Val Gly Ala Ile Pro Val Asp Leu Lys
945                 950                 955                 960

Arg Arg Leu Asp Ser Ile Thr Ser Ser Gln Ser Ser Ala Ser Ser Gly
                965                 970                 975

Phe Val Glu Glu Lys Ser Leu Ser Asp Val Glu Glu Glu Glu Ala Pro
            980                 985                 990

Glu Asp Leu Tyr Lys Asp Phe Leu  Thr Leu Glu His Leu  Ile Cys Tyr
        995                 1000                1005

Ser Phe  Gln Val Ala Lys Gly  Met Glu Phe Leu Ala  Ser Arg Lys
    1010                1015                1020

Cys Ile  His Arg Asp Leu Ala  Ala Arg Asn Ile Leu  Leu Ser Glu
    1025                1030                1035

Lys Asn  Val Val Lys Ile Cys  Asp Phe Gly Leu Ala  Arg Asp Ile
    1040                1045                1050

Tyr Lys  Asp Pro Asp Tyr Val  Arg Lys Gly Asp Ala  Arg Leu Pro
    1055                1060                1065

Leu Lys  Trp Met Ala Pro Glu  Thr Ile Phe Asp Arg  Val Tyr Thr
    1070                1075                1080

Ile Gln  Ser Asp Val Trp Ser  Phe Gly Val Leu Leu  Trp Glu Ile
    1085                1090                1095

Phe Ser  Leu Gly Ala Ser Pro  Tyr Pro Gly Val Lys  Ile Asp Glu
    1100                1105                1110

Glu Phe  Cys Arg Arg Leu Lys  Glu Gly Thr Arg Met  Arg Ala Pro
    1115                1120                1125

Asp Tyr  Thr Thr Pro Glu Met  Tyr Gln Thr Met Leu  Asp Cys Trp
    1130                1135                1140

His Gly  Glu Pro Ser Gln Arg  Pro Thr Phe Ser Glu  Leu Val Glu
    1145                1150                1155

His Leu  Gly Asn Leu Leu Gln  Ala Asn Ala Gln Gln  Asp Gly Lys
    1160                1165                1170

Asp Tyr  Ile Val Leu Pro Ile  Ser Glu Thr Leu Ser  Met Glu Glu
    1175                1180                1185

Asp Ser  Gly Leu Ser Leu Pro  Thr Ser Pro Val Ser  Cys Met Glu
    1190                1195                1200

Glu Glu  Glu Val Cys Asp Pro  Lys Phe His Tyr Asp  Asn Thr Ala
    1205                1210                1215

Gly Ile  Ser Gln Tyr Leu Gln  Asn Ser Lys Arg Lys  Ser Arg Pro
    1220                1225                1230

Val Ser  Val Lys Thr Phe Glu  Asp Ile Pro Leu Glu  Glu Pro Glu
    1235                1240                1245

Val Lys  Val Ile Pro Asp Asp  Asn Gln Thr Asp Ser  Gly Met Val
    1250                1255                1260
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Leu Ala  Ser Glu Glu Leu Lys  Thr Leu Glu Asp Arg  Thr Lys Leu
    1265                 1270                 1275

Ser Pro  Ser Phe Gly Gly Met  Val Pro Ser Lys Ser  Arg Glu Ser
    1280                 1285                 1290

Val Ala  Ser Glu Gly Ser Asn  Gln Thr Ser Gly Tyr  Gln Ser Gly
    1295                 1300                 1305

Tyr His  Ser Asp Asp Thr Asp  Thr Thr Val Tyr Ser  Ser Glu Glu
    1310                 1315                 1320

Ala Glu  Leu Leu Lys Leu Ile  Glu Ile Gly Val Gln  Thr Gly Ser
    1325                 1330                 1335

Thr Ala  Gln Ile Leu Gln Pro  Asp Ser Gly Thr Thr  Leu Ser Ser
    1340                 1345                 1350

Pro Pro  Val
    1355


<210> SEQ ID NO 40
<211> LENGTH: 470
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 40

Met Glu Phe Gly Leu Ser Trp Leu Phe Leu Val Ala Ile Leu Lys Gly
1               5                  10                  15

Val Gln Cys Glu Val Gln Leu Val Glu Thr Gly Gly Gly Leu Val Gln
            20                  25                  30

Pro Gly Asn Ser Leu Lys Leu Ser Cys Ala Thr Ser Gly Phe Ile Phe
        35                  40                  45

Ser Thr Thr Trp Met Asn Trp Ile Arg Gln Thr Pro Gly Lys Arg Leu
    50                  55                  60

Glu Trp Leu Ala Gln Ile Glu Asp Lys Ser Asn Asn Tyr Phe Ile Ser
65                  70                  75                  80

Tyr Ser Glu Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser
                85                  90                  95

Lys Ser Ser Val Tyr Leu Gln Met Asn Asn Leu Lys Glu Glu Asp Thr
            100                 105                 110

Ala Ile Tyr Tyr Cys Ser Trp Lys Tyr Arg Ser Asn Tyr Tyr Phe Asp
        115                 120                 125

Tyr Trp Gly Gln Gly Val Met Val Thr Val Ser Ser Ala Ser Thr Lys
    130                 135                 140

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
145                 150                 155                 160

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
                165                 170                 175

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
            180                 185                 190

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
        195                 200                 205

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
    210                 215                 220

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro
225                 230                 235                 240
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Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
                245                 250                 255

Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
            260                 265                 270

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
        275                 280                 285

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
    290                 295                 300

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
305                 310                 315                 320

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
                325                 330                 335

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
            340                 345                 350

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
        355                 360                 365

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
    370                 375                 380

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
385                 390                 395                 400

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
                405                 410                 415

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
            420                 425                 430

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
        435                 440                 445

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
    450                 455                 460

Ser Leu Ser Pro Gly Lys
465                 470


<210> SEQ ID NO 41
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
<220> FEATURE:
<221> NAME/KEY: X
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: I or M

<400> SEQUENCE: 41

Xaa Tyr Gly Met Ser
1               5


<210> SEQ ID NO 42
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
<220> FEATURE:
<221> NAME/KEY: X
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: V or I
<220> FEATURE:
<221> NAME/KEY: X
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<222> LOCATION: (16)..(16)
<223> OTHER INFORMATION: E or K

<400> SEQUENCE: 42

Ser Ile Ser Xaa Gly Gly Ser Tyr Thr Tyr Tyr Ala Asp Ser Val Xaa
1 5 10 15

Gly

<210> SEQ ID NO 43
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
<220> FEATURE:
<221> NAME/KEY: X
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: L or M

<400> SEQUENCE: 43

Glu Xaa Asp Gly Asn Tyr Asp Tyr
1 5

<210> SEQ ID NO 44
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
<220> FEATURE:
<221> NAME/KEY: X
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: T or S

<400> SEQUENCE: 44

Xaa Tyr Trp Ile Met
1 5

<210> SEQ ID NO 45
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
<220> FEATURE:
<221> NAME/KEY: X
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: T or S

<400> SEQUENCE: 45

Asp Ile Tyr Pro Gly Xaa Gly Ser Thr Asn Tyr Asn Glu Lys Phe Lys
1 5 10 15

Ser

<210> SEQ ID NO 46
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 46

Asp Ser Asn Pro Asp Tyr
1 5

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<210> SEQ ID NO 47
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
<220> FEATURE:
<221> NAME/KEY: X
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: D or E
<220> FEATURE:
<221> NAME/KEY: X
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: T or H

<400> SEQUENCE: 47

Arg Ala Ser Glu Ser Val Xaa Asn Ser Gly Ile Ser Phe Met Xaa
1 5 10 15

<210> SEQ ID NO 48
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
<220> FEATURE:
<221> NAME/KEY: X
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: T or Y
<220> FEATURE:
<221> NAME/KEY: X
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: G or R

<400> SEQUENCE: 48

Ala Ala Ser Xaa Gln Xaa Ser
1 5

<210> SEQ ID NO 49
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 49

Gln Gln Ser Lys Glu Val Pro Tyr Thr
1 5

<210> SEQ ID NO 50
<211> LENGTH: 120
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 50

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Ser
20 25 30

Trp Met Asn Trp Val Lys Gln Arg Pro Gly Glu Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Tyr Tyr Asn Gly Lys Phe
50 55 60

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Gln Val Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
                85                  90                  95

Ala Ile Phe Leu Asp Thr Ser Gly Arg Tyr Val Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Thr Leu Thr Ile Ser Ser
        115                 120


<210> SEQ ID NO 51
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 51

Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val Gly
1               5                   10                  15

Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Asn Thr Ala
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ser Ala Ser Tyr Arg Tyr Ile Gly Val Pro Asp Arg Phe Thr Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Val Gln Ser
65                  70                  75                  80

Glu Asp Leu Thr Val Tyr Tyr Cys Gln Gln His Tyr Arg Ala Pro Leu
                85                  90                  95

Thr Phe Gly Ser Gly Thr Lys Leu Glu Leu Lys
            100                 105


<210> SEQ ID NO 52
<211> LENGTH: 115
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 52

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Asn Thr Tyr
            20                  25                  30

Trp Ile Met Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Asp Ile Tyr Pro Gly Thr Gly Ser Thr Asn Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Ser Lys Val Thr Leu Thr Ala Asp Thr Ser Ser Thr Thr Ala Tyr
65                  70                  75                  80

Met Gln Val Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Gly Arg Asp Ser Asn Pro Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr
            100                 105                 110

Val Ser Ser
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115

<210> SEQ ID NO 53
<211> LENGTH: 111
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 53

Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Asn Ser
20 25 30

Gly Ile Ser Phe Met Thr Trp Phe Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Tyr Ala Ala Ser Thr Gln Gly Ser Gly Val Pro Ala
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His
65 70 75 80

Pro Val Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln Gln Ser Lys
85 90 95

Glu Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> SEQ ID NO 54
<211> LENGTH: 115
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 54

Gln Ala Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Thr
1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Asn Ser Tyr
20 25 30

Trp Ile Met Trp Val Lys Gln Ser Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Asp Ile Tyr Pro Gly Ser Gly Ser Thr Asn Tyr Asn Glu Lys Phe
50 55 60

Lys Ser Lys Val Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Val Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Ser Asn Pro Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr
100 105 110

Val Ser Ser
115

<210> SEQ ID NO 55
<211> LENGTH: 111
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 55

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Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1               5                   10                  15

Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Glu Asn Ser
            20                  25                  30

Gly Ile Ser Phe Met His Trp Phe Gln Gln Lys Pro Gly Gln Pro Pro
        35                  40                  45

Lys Leu Leu Ile Tyr Ala Ala Ser Tyr Gln Arg Ser Gly Val Pro Ala
    50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His
65                  70                  75                  80

Pro Val Glu Glu Asp Asp Ile Ala Met Tyr Phe Cys Gln Gln Ser Lys
                85                  90                  95

Glu Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110


<210> SEQ ID NO 56
<211> LENGTH: 123
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 56

Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Ser Leu Ser Cys Ala Val Ser Gly Phe Thr Phe Thr Asp Tyr
            20                  25                  30

Tyr Met Ser Trp Val Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu
        35                  40                  45

Gly Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Glu Tyr Ser Ala
    50                  55                  60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gln Ser Ile
65                  70                  75                  80

Leu Tyr Leu Gln Met Asn Ala Leu Arg Ala Glu Asp Ser Ala Thr Tyr
                85                  90                  95

Tyr Cys Ala Arg Phe Asp Tyr Tyr Gly Ser Thr Tyr Cys Phe Asp Tyr
            100                 105                 110

Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
        115                 120


<210> SEQ ID NO 57
<211> LENGTH: 111
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 57

Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1               5                   10                  15

Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Gln Ser Val Ser Thr Ser
            20                  25                  30

Ser Ser Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
        35                  40                  45

Lys Leu Leu Ile Lys Tyr Ala Ser Asn Leu Glu Ser Gly Val Pro Ala
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    50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65                  70                  75                  80

Pro Val Glu Glu Glu Asp Thr Ala Thr Tyr Tyr Cys Gln His Thr Trp
                85                  90                  95

Glu Ile Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
            100                 105                 110


<210> SEQ ID NO 58
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 58

Glu Val Gln Leu Val Glu Ser Gly Gly Asp Leu Val Lys Pro Gly Gly
1               5                   10                  15

Ser Leu Lys Leu Ser Cys Thr Ala Ser Gly Phe Ser Phe Ser Ile Tyr
            20                  25                  30

Gly Met Ser Trp Val Arg Gln Thr Pro Asp Lys Arg Leu Glu Trp Val
        35                  40                  45

Ala Ser Ile Ser Val Gly Gly Ser Tyr Thr Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Glu Gly Arg Phe Thr Ile Ser Arg Glu Asn Ala Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Lys Ser Glu Asp Thr Ala Leu Tyr Tyr Cys
                85                  90                  95

Ala Arg Glu Leu Asp Gly Asn Tyr Asp Tyr Trp Gly Gln Gly Thr Ser
            100                 105                 110

Leu Thr Val Ser Ser
        115


<210> SEQ ID NO 59
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 59

Asp Ile Val Ile Thr Gln Asn Glu Leu Ser Asn Pro Val Thr Phe Gly
1               5                   10                  15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu Tyr Lys
            20                  25                  30

Asp Gly Lys Thr Tyr Leu Asn Trp Phe Leu Gln Arg Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Leu Met Ser Thr Arg Ala Ser Gly Val Ser
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Glu Ile
65                  70                  75                  80

Ser Arg Val Lys Ala Glu Asp Val Gly Val Tyr Tyr Cys Gln Gln Leu
                85                  90                  95

Val Glu Tyr Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110
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<210> SEQ ID NO 60
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 60

Glu Val Gln Leu Val Glu Ser Gly Gly Asp Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Met Tyr
20 25 30

Gly Met Ser Trp Val Arg Gln Thr Pro Asp Lys Arg Leu Glu Trp Val
35 40 45

Ala Ser Ile Ser Ile Gly Gly Ser Tyr Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Glu Gly Arg Phe Thr Ile Ser Arg Glu Asn Ala Lys Asn Thr Leu Phe
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Ser Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Glu Met Asp Gly Asn Tyr Asp Tyr Trp Gly His Gly Thr Thr
100 105 110

Leu Thr Val Ser Ser
115

<210> SEQ ID NO 61
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 61

Asp Val Met Ile Thr Gln Asp Glu Leu Ser Asn Pro Val Thr Phe Gly
1 5 10 15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu Tyr Lys
20 25 30

Asp Gly Lys Thr Tyr Leu Asn Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Met Ser Thr Arg Ala Ser Gly Val Ser
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Glu Ile
65 70 75 80

Ser Arg Val Lys Ala Glu Asp Val Gly Ile Tyr Tyr Cys Gln Gln Leu
85 90 95

Val Glu Tyr Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> SEQ ID NO 62
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 62

Glu Val Gln Leu Val Glu Ser Gly Gly Asp Leu Val Lys Pro Gly Gly

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1               5                   10                  15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Met Tyr
            20                  25                  30

Gly Met Ser Trp Val Arg Gln Thr Pro Asp Lys Arg Leu Glu Trp Val
        35                  40                  45

Ala Ser Ile Ser Ile Gly Gly Ser Tyr Thr Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Glu Gly Arg Phe Ile Ile Ser Arg Glu Asn Ala Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Lys Ser Glu Asp Thr Ala Leu Tyr Tyr Cys
                85                  90                  95

Ala Arg Glu Leu Asp Gly Asn Tyr Asp Tyr Trp Gly Gln Gly Thr Thr
            100                 105                 110

Leu Thr Val Ser Ser
        115


<210> SEQ ID NO 63
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 63

His Ile Met Ile Thr Gln Asp Glu Leu Ser Asn Pro Val Thr Phe Gly
1               5                   10                  15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu Tyr Lys
            20                  25                  30

Asp Gly Lys Thr Tyr Leu Asn Trp Phe Leu Gln Arg Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Leu Met Ser Thr Arg Ala Ser Gly Val Ser
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Glu Ile
65                  70                  75                  80

Ser Arg Val Lys Ala Glu Asp Val Gly Ile Tyr Tyr Cys Gln Gln Leu
                85                  90                  95

Val Glu Tyr Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110


<210> SEQ ID NO 64
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 64

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1               5                   10                  15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
            20                  25                  30


<210> SEQ ID NO 65
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 65

Trp Val Arg Gln Thr Pro Gly Lys Arg Leu Glu Trp Val Ala
1 5 10

<210> SEQ ID NO 66
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 66

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> SEQ ID NO 67
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 67

Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
1 5 10

<210> SEQ ID NO 68
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 68

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
20 25 30

<210> SEQ ID NO 69
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 69

Trp Val Arg Gln Ala Pro Gly Lys Arg Leu Glu Trp Val Ala
1 5 10

<210> SEQ ID NO 70
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 70

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln

-continued

1 5 10 15

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> SEQ ID NO 71
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 71

Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
1 5 10

<210> SEQ ID NO 72
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 72

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
20 25 30

<210> SEQ ID NO 73
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 73

Trp Val Arg Gln Ala Pro Gly Lys Arg Leu Glu Trp Val Ala
1 5 10

<210> SEQ ID NO 74
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 74

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> SEQ ID NO 75
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 75

Trp Gly His Gly Thr Thr Leu Thr Val Ser Ser
1 5 10

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<210> SEQ ID NO 76
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 76

Asp Ile Val Ile Thr Gln Asp Glu Leu Ser Leu Pro Val Thr Phe Gly
1 5 10 15

Glu Ser Val Ser Ile Ser Cys
20

<210> SEQ ID NO 77
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 77

Trp Phe Leu Gln Arg Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr
1 5 10 15

<210> SEQ ID NO 78
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 78

Gly Val Ser Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Ile Tyr Tyr Cys
20 25 30

<210> SEQ ID NO 79
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 79

Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
1 5 10

<210> SEQ ID NO 80
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 80

Asp Ile Val Ile Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Ser Val Ser Ile Ser Cys
20

<210> SEQ ID NO 81

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<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 81

Trp Phe Leu Gln Arg Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr
1 5 10 15

<210> SEQ ID NO 82
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 82

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys
20 25 30

<210> SEQ ID NO 83
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 83

Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
1 5 10

<210> SEQ ID NO 84
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
<220> FEATURE:
<221> NAME/KEY: X
<222> LOCATION: (19)..(19)
<223> OTHER INFORMATION: R or K

<400> SEQUENCE: 84

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Xaa Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
20 25 30

<210> SEQ ID NO 85
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
<220> FEATURE:
<221> NAME/KEY: X
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: A or T

<400> SEQUENCE: 85

Trp Val Arg Gln Xaa Pro Gly Lys Arg Leu Glu Trp Val Ala
1 5 10

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<210> SEQ ID NO 86
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
<220> FEATURE:
<221> NAME/KEY: X
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: R or K

<400> SEQUENCE: 86

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Xaa Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> SEQ ID NO 87
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
<220> FEATURE:
<221> NAME/KEY: X
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Q or H

<400> SEQUENCE: 87

Trp Gly Xaa Gly Thr Thr Leu Thr Val Ser Ser
1 5 10

<210> SEQ ID NO 88
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
<220> FEATURE:
<221> NAME/KEY: X
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: D or T
<220> FEATURE:
<221> NAME/KEY: X
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: E or P
<220> FEATURE:
<221> NAME/KEY: X
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: F or P

<400> SEQUENCE: 88

Asp Ile Val Ile Thr Gln Xaa Xaa Leu Ser Leu Pro Val Thr Xaa Gly
1 5 10 15

Glu Ser Val Ser Ile Ser Cys
20

<210> SEQ ID NO 89
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 89

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Trp Phe Leu Gln Arg Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr
1 5 10 15

<210> SEQ ID NO 90
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
<220> FEATURE:
<221> NAME/KEY: X
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: S or P
<220> FEATURE:
<221> NAME/KEY: X
<222> LOCATION: (29)..(29)
<223> OTHER INFORMATION: V or I

<400> SEQUENCE: 90

Gly Val Xaa Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Xaa Tyr Tyr Cys
20 25 30

<210> SEQ ID NO 91
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 91

Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
1 5 10

<210> SEQ ID NO 92
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 92

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Ser Ser Ser Ser Tyr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Leu Asp Gly Asn Tyr Asp Tyr Trp Gly Gln Gly Thr Thr
100 105 110

Leu Thr Val Ser Ser
115

<210> SEQ ID NO 93

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<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 93

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Met Tyr
20 25 30

Gly Met Ser Trp Val Arg Gln Thr Pro Gly Lys Arg Leu Glu Trp Val
35 40 45

Ala Ser Ile Ser Ile Gly Gly Ser Tyr Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Leu Asp Gly Asn Tyr Asp Tyr Trp Gly Gln Gly Thr Thr
100 105 110

Leu Thr Val Ser Ser
115

<210> SEQ ID NO 94
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 94

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Met Tyr
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Arg Leu Glu Trp Val
35 40 45

Ala Ser Ile Ser Ile Gly Gly Ser Tyr Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Leu Asp Gly Asn Tyr Asp Tyr Trp Gly Gln Gly Thr Thr
100 105 110

Leu Thr Val Ser Ser
115

<210> SEQ ID NO 95
<211> LENGTH: 101
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 95

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Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1               5                   10                  15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu Asp Ser
            20                  25                  30

Asp Asp Gly Asn Thr Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln
        35                  40                  45

Ser Pro Gln Leu Leu Ile Tyr Thr Leu Ser Tyr Arg Ala Ser Gly Val
    50                  55                  60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys
65                  70                  75                  80

Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln
                85                  90                  95

Arg Ile Glu Phe Pro
            100


<210> SEQ ID NO 96
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 96

Asp Ile Val Ile Thr Gln Asp Glu Leu Ser Leu Pro Val Thr Phe Gly
1               5                   10                  15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu Tyr Lys
            20                  25                  30

Asp Gly Lys Thr Tyr Leu Asn Trp Phe Leu Gln Arg Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Leu Met Ser Thr Arg Ala Ser Gly Val Ser
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Ile Tyr Tyr Cys Gln Gln Leu
                85                  90                  95

Val Glu Tyr Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110


<210> SEQ ID NO 97
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 97

Asp Ile Val Ile Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1               5                   10                  15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu Tyr Lys
            20                  25                  30

Asp Gly Lys Thr Tyr Leu Asn Trp Phe Leu Gln Arg Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Leu Met Ser Thr Arg Ala Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80
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Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Gln Gln Leu
85 90 95

Val Glu Tyr Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> SEQ ID NO 98
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 98

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Met Tyr
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Arg Leu Glu Trp Val
35 40 45

Ala Ser Ile Ser Ile Gly Gly Ser Tyr Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Met Asp Gly Asn Tyr Asp Tyr Trp Gly His Gly Thr Thr
100 105 110

Leu Thr Val Ser Ser
115

<210> SEQ ID NO 99
<211> LENGTH: 239
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 99

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Phe Pro Gly Ser Arg Cys Asp Ile Val Leu Thr Gln Ser Pro Ala Leu
20 25 30

Ala Val Ser Leu Glu Gln Arg Ala Thr Ile Ser Cys Lys Thr Ser Gln
35 40 45

Asn Val Asp Tyr Tyr Gly Ile Ser Tyr Leu His Trp Tyr Gln Gln Lys
50 55 60

Pro Gly Gln Gln Pro Lys Leu Leu Ile Tyr Glu Gly Ser Asn Leu Ala
65 70 75 80

Ser Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
85 90 95

Thr Leu Thr Ile Asp Pro Val Glu Ala Asp Asp Ile Val Thr Tyr Tyr
100 105 110

Cys Gln Gln Ser Lys Asp Tyr Pro Tyr Thr Phe Gly Ala Gly Thr Lys
115 120 125

Leu Glu Leu Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro

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    130                 135                 140

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
145                 150                 155                 160

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
                165                 170                 175

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
            180                 185                 190

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
        195                 200                 205

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
    210                 215                 220

Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225                 230                 235


<210> SEQ ID NO 100
<211> LENGTH: 1367
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 100

Met Glu Ser Lys Ala Leu Leu Ala Val Ala Leu Trp Phe Cys Val Glu
1               5                   10                  15

Thr Arg Ala Ala Ser Val Gly Leu Pro Gly Asp Phe Leu His Pro Pro
            20                  25                  30

Lys Leu Ser Thr Gln Lys Asp Ile Leu Thr Ile Leu Ala Asn Thr Thr
        35                  40                  45

Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu Asp Trp Leu Trp Pro
    50                  55                  60

Asn Ala Gln Arg Asp Ser Glu Glu Arg Val Leu Val Thr Glu Cys Gly
65                  70                  75                  80

Gly Gly Asp Ser Ile Phe Cys Lys Thr Leu Thr Ile Pro Arg Val Val
                85                  90                  95

Gly Asn Asp Thr Gly Ala Tyr Lys Cys Ser Tyr Arg Asp Val Asp Ile
            100                 105                 110

Ala Ser Thr Val Tyr Val Tyr Val Arg Asp Tyr Arg Ser Pro Phe Ile
        115                 120                 125

Ala Ser Val Ser Asp Gln His Gly Ile Val Tyr Ile Thr Glu Asn Lys
    130                 135                 140

Asn Lys Thr Val Val Ile Pro Cys Arg Gly Ser Ile Ser Asn Leu Asn
145                 150                 155                 160

Val Ser Leu Cys Ala Arg Tyr Pro Glu Lys Arg Phe Val Pro Asp Gly
                165                 170                 175

Asn Arg Ile Ser Trp Asp Ser Glu Ile Gly Phe Thr Leu Pro Ser Tyr
            180                 185                 190

Met Ile Ser Tyr Ala Gly Met Val Phe Cys Glu Ala Lys Ile Asn Asp
        195                 200                 205

Glu Thr Tyr Gln Ser Ile Met Tyr Ile Val Val Val Val Gly Tyr Arg
    210                 215                 220

Ile Tyr Asp Val Ile Leu Ser Pro Pro His Glu Ile Glu Leu Ser Ala
225                 230                 235                 240

Gly Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val
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               245                 250                 255

Gly Leu Asp Phe Thr Trp His Ser Pro Pro Ser Lys Ser His His Lys
            260                 265                 270

Lys Ile Val Asn Arg Asp Val Lys Pro Phe Pro Gly Thr Val Ala Lys
        275                 280                 285

Met Phe Leu Ser Thr Leu Thr Ile Glu Ser Val Thr Lys Ser Asp Gln
    290                 295                 300

Gly Glu Tyr Thr Cys Val Ala Ser Ser Gly Arg Met Ile Lys Arg Asn
305                 310                 315                 320

Arg Thr Phe Val Arg Val His Thr Lys Pro Phe Ile Ala Phe Gly Ser
                325                 330                 335

Gly Met Lys Ser Leu Val Glu Ala Thr Val Gly Ser Gln Val Arg Ile
            340                 345                 350

Pro Val Lys Tyr Leu Ser Tyr Pro Ala Pro Asp Ile Lys Trp Tyr Arg
        355                 360                 365

Asn Gly Arg Pro Ile Glu Ser Asn Tyr Thr Met Ile Val Gly Asp Glu
    370                 375                 380

Leu Thr Ile Met Glu Val Thr Glu Arg Asp Ala Gly Asn Tyr Thr Val
385                 390                 395                 400

Ile Leu Thr Asn Pro Ile Ser Met Glu Lys Gln Ser His Met Val Ser
                405                 410                 415

Leu Val Val Asn Val Pro Pro Gln Ile Gly Glu Lys Ala Leu Ile Ser
            420                 425                 430

Pro Met Asp Ser Tyr Gln Tyr Gly Thr Met Gln Thr Leu Thr Cys Thr
        435                 440                 445

Val Tyr Ala Asn Pro Pro Leu His His Ile Gln Trp Tyr Trp Gln Leu
    450                 455                 460

Glu Glu Ala Cys Ser Tyr Arg Pro Gly Gln Thr Ser Pro Tyr Ala Cys
465                 470                 475                 480

Lys Glu Trp Arg His Val Glu Asp Phe Gln Gly Gly Asn Lys Ile Glu
                485                 490                 495

Val Thr Lys Asn Gln Tyr Ala Leu Ile Glu Gly Lys Asn Lys Thr Val
            500                 505                 510

Ser Thr Leu Val Ile Gln Ala Ala Asn Val Ser Ala Leu Tyr Lys Cys
        515                 520                 525

Glu Ala Ile Asn Lys Ala Gly Arg Gly Glu Arg Val Ile Ser Phe His
    530                 535                 540

Val Ile Arg Gly Pro Glu Ile Thr Val Gln Pro Ala Ala Gln Pro Thr
545                 550                 555                 560

Glu Gln Glu Ser Val Ser Leu Leu Cys Thr Ala Asp Arg Asn Thr Phe
                565                 570                 575

Glu Asn Leu Thr Trp Tyr Lys Leu Gly Ser Gln Ala Thr Ser Val His
            580                 585                 590

Met Gly Glu Ser Leu Thr Pro Val Cys Lys Asn Leu Asp Ala Leu Trp
        595                 600                 605

Lys Leu Asn Gly Thr Met Phe Ser Asn Ser Thr Asn Asp Ile Leu Ile
    610                 615                 620

Val Ala Phe Gln Asn Ala Ser Leu Gln Asp Gln Gly Asp Tyr Val Cys
625                 630                 635                 640

Ser Ala Gln Asp Lys Lys Thr Lys Lys Arg His Cys Leu Val Lys Gln
                645                 650                 655
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Leu Ile Ile Leu Glu Arg Met Ala Pro Met Ile Thr Gly Asn Leu Glu
            660                 665                 670

Asn Gln Thr Thr Thr Ile Gly Glu Thr Ile Glu Val Thr Cys Pro Ala
        675                 680                 685

Ser Gly Asn Pro Thr Pro His Ile Thr Trp Phe Lys Asp Asn Glu Thr
    690                 695                 700

Leu Val Glu Asp Ser Gly Ile Val Leu Arg Asp Gly Asn Arg Asn Leu
705                 710                 715                 720

Thr Ile Arg Arg Val Arg Lys Glu Asp Gly Gly Leu Tyr Thr Cys Gln
                725                 730                 735

Ala Cys Asn Val Leu Gly Cys Ala Arg Ala Glu Thr Leu Phe Ile Ile
            740                 745                 750

Glu Gly Ala Gln Glu Lys Thr Asn Leu Glu Val Ile Ile Leu Val Gly
        755                 760                 765

Thr Ala Val Ile Ala Met Phe Phe Trp Leu Leu Leu Val Ile Leu Val
    770                 775                 780

Arg Thr Val Lys Arg Ala Asn Glu Gly Glu Leu Lys Thr Gly Tyr Leu
785                 790                 795                 800

Ser Ile Val Met Asp Pro Asp Glu Leu Pro Leu Asp Glu Arg Cys Glu
                805                 810                 815

Arg Leu Pro Tyr Asp Ala Ser Lys Trp Glu Phe Pro Arg Asp Arg Leu
            820                 825                 830

Lys Leu Gly Lys Pro Leu Gly Arg Gly Ala Phe Gly Gln Val Ile Glu
        835                 840                 845

Ala Asp Ala Phe Gly Ile Asp Lys Thr Ala Thr Cys Lys Thr Val Ala
    850                 855                 860

Val Lys Met Leu Lys Glu Gly Ala Thr His Ser Glu His Arg Ala Leu
865                 870                 875                 880

Met Ser Glu Leu Lys Ile Leu Ile His Ile Gly His His Leu Asn Val
                885                 890                 895

Val Asn Leu Leu Gly Ala Cys Thr Lys Pro Gly Gly Pro Leu Met Val
            900                 905                 910

Ile Val Glu Phe Ser Lys Phe Gly Asn Leu Ser Thr Tyr Leu Arg Gly
        915                 920                 925

Lys Arg Asn Glu Phe Val Pro Tyr Lys Ser Lys Gly Ala Arg Phe Arg
    930                 935                 940

Gln Gly Lys Asp Tyr Val Gly Glu Leu Ser Val Asp Leu Lys Arg Arg
945                 950                 955                 960

Leu Asp Ser Ile Thr Ser Ser Gln Ser Ser Ala Ser Ser Gly Phe Val
                965                 970                 975

Glu Glu Lys Ser Leu Ser Asp Val Glu Glu Glu Glu Ala Ser Glu Glu
            980                 985                 990

Leu Tyr Lys Asp Phe Leu Thr Leu  Glu His Leu Ile Cys  Tyr Ser Phe
        995                 1000                1005

Gln Val  Ala Lys Gly Met Glu  Phe Leu Ala Ser Arg  Lys Cys Ile
    1010                1015                1020

His Arg  Asp Leu Ala Ala Arg  Asn Ile Leu Leu Ser  Glu Lys Asn
    1025                1030                1035

Val Val  Lys Ile Cys Asp Phe  Gly Leu Ala Arg Asp  Ile Tyr Lys
    1040                1045                1050
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Asp Pro  Asp Tyr Val Arg Lys  Gly Asp Ala Arg Leu  Pro Leu Lys
    1055                 1060                 1065

Trp Met  Ala Pro Glu Thr Ile  Phe Asp Arg Val Tyr  Thr Ile Gln
    1070                 1075                 1080

Ser Asp  Val Trp Ser Phe Gly  Val Leu Leu Trp Glu  Ile Phe Ser
    1085                 1090                 1095

Leu Gly  Ala Ser Pro Tyr Pro  Gly Val Lys Ile Asp  Glu Glu Phe
    1100                 1105                 1110

Cys Arg  Arg Leu Lys Glu Gly  Thr Arg Met Arg Ala  Pro Asp Tyr
    1115                 1120                 1125

Thr Thr  Pro Glu Met Tyr Gln  Thr Met Leu Asp Cys  Trp His Glu
    1130                 1135                 1140

Asp Pro  Asn Gln Arg Pro Ser  Phe Ser Glu Leu Val  Glu His Leu
    1145                 1150                 1155

Gly Asn  Leu Leu Gln Ala Asn  Ala Gln Gln Asp Gly  Lys Asp Tyr
    1160                 1165                 1170

Ile Val  Leu Pro Met Ser Glu  Thr Leu Ser Met Glu  Glu Asp Ser
    1175                 1180                 1185

Gly Leu  Ser Leu Pro Thr Ser  Pro Val Ser Cys Met  Glu Glu Glu
    1190                 1195                 1200

Glu Val  Cys Asp Pro Lys Phe  His Tyr Asp Asn Thr  Ala Gly Ile
    1205                 1210                 1215

Ser His  Tyr Leu Gln Asn Ser  Lys Arg Lys Ser Arg  Pro Val Ser
    1220                 1225                 1230

Val Lys  Thr Phe Glu Asp Ile  Pro Leu Glu Glu Pro  Glu Val Lys
    1235                 1240                 1245

Val Ile  Pro Asp Asp Ser Gln  Thr Asp Ser Gly Met  Val Leu Ala
    1250                 1255                 1260

Ser Glu  Glu Leu Lys Thr Leu  Glu Asp Arg Asn Lys  Leu Ser Pro
    1265                 1270                 1275

Ser Phe  Gly Gly Met Met Pro  Ser Lys Ser Arg Glu  Ser Val Ala
    1280                 1285                 1290

Ser Glu  Gly Ser Asn Gln Thr  Ser Gly Tyr Gln Ser  Gly Tyr His
    1295                 1300                 1305

Ser Asp  Asp Thr Asp Thr Thr  Val Tyr Ser Ser Asp  Glu Ala Gly
    1310                 1315                 1320

Leu Leu  Lys Met Val Asp Ala  Ala Val His Ala Asp  Ser Gly Thr
    1325                 1330                 1335

Thr Leu  Gln Leu Thr Ser Cys  Leu Asn Gly Ser Gly  Pro Val Pro
    1340                 1345                 1350

Ala Pro  Pro Pro Thr Pro Gly  Asn His Glu Arg Gly  Ala Ala
    1355                 1360                 1365


<210> SEQ ID NO 101
<211> LENGTH: 1356
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 101

Met Ala Ser Lys Val Leu Leu Ala Val Ala Leu Trp Leu Cys Val Glu
1               5                  10                  15
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Thr Arg Ala Ala Ser Val Gly Leu Pro Ser Val Ser Leu Asp Leu Pro
            20                  25                  30

Arg Leu Ser Ile Gln Lys Asp Ile Leu Thr Ile Lys Ala Asn Thr Thr
        35                  40                  45

Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu Asp Trp Leu Trp Pro
    50                  55                  60

Asn Asn Gln Ser Gly Ser Glu Gln Arg Val Glu Val Thr Glu Cys Ser
65                  70                  75                  80

Asp Gly Leu Phe Cys Lys Thr Leu Thr Ile Pro Lys Val Ile Gly Asn
                85                  90                  95

Asp Thr Gly Ala Tyr Lys Cys Phe Tyr Arg Glu Thr Asp Leu Ala Ser
            100                 105                 110

Val Ile Tyr Val Tyr Val Gln Asp Tyr Arg Ser Pro Phe Ile Ala Ser
        115                 120                 125

Val Ser Asp Gln His Gly Val Val Tyr Ile Thr Glu Asn Lys Asn Lys
    130                 135                 140

Thr Val Val Ile Pro Cys Leu Gly Ser Ile Ser Asn Leu Asn Val Ser
145                 150                 155                 160

Leu Cys Ala Arg Tyr Pro Glu Lys Arg Phe Val Pro Asp Gly Asn Arg
                165                 170                 175

Ile Ser Trp Asp Ser Lys Lys Gly Phe Thr Ile Pro Ser Tyr Met Ile
            180                 185                 190

Ser Tyr Ala Gly Met Val Phe Cys Glu Ala Lys Ile Asn Asp Glu Ser
        195                 200                 205

Tyr Gln Ser Ile Met Tyr Ile Val Val Val Val Gly Tyr Arg Ile Tyr
    210                 215                 220

Asp Val Val Leu Ser Pro Ser His Gly Val Glu Leu Ser Val Gly Glu
225                 230                 235                 240

Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile
                245                 250                 255

Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu
            260                 265                 270

Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe
        275                 280                 285

Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu
    290                 295                 300

Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr
305                 310                 315                 320

Phe Val Arg Val His Glu Lys Pro Phe Val Ala Phe Gly Ser Gly Met
                325                 330                 335

Glu Ser Leu Val Glu Ala Thr Val Gly Glu Arg Val Arg Ile Pro Val
            340                 345                 350

Lys Tyr Leu Gly Tyr Pro Pro Pro Glu Ile Lys Trp Tyr Lys Asn Gly
        355                 360                 365

Ile Pro Leu Glu Ser Asn His Thr Val Lys Val Gly His Val Leu Thr
    370                 375                 380

Ile Met Glu Val Ser Glu Arg Asp Thr Gly Asn Tyr Thr Val Ile Leu
385                 390                 395                 400

Thr Asn Pro Ile Ser Lys Glu Lys Gln Ser His Val Val Ser Leu Val
                405                 410                 415

Val Tyr Val Pro Pro Gln Ile Gly Glu Lys Ser Leu Ile Ser Pro Val
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            420                 425                 430

Asp Ser Tyr Gln Tyr Gly Thr Thr Gln Thr Leu Thr Cys Thr Val Tyr
        435                 440                 445

Ala Ile Pro Pro Pro His His Ile His Trp Tyr Trp Gln Leu Glu Glu
    450                 455                 460

Glu Cys Pro Asn Glu Pro Ser Gln Ala Val Ser Val Thr Asn Pro Tyr
465                 470                 475                 480

Pro Cys Glu Glu Trp Arg Ser Val Glu Asp Phe Gln Gly Gly Asn Lys
                485                 490                 495

Ile Glu Val Asn Lys Asn Gln Phe Ala Leu Ile Glu Gly Lys Asn Lys
            500                 505                 510

Thr Val Ser Thr Leu Val Ile Gln Ala Ala Asn Val Ser Ala Leu Tyr
        515                 520                 525

Lys Cys Glu Ala Val Asn Lys Val Gly Arg Gly Glu Arg Val Ile Ser
    530                 535                 540

Phe His Val Thr Arg Gly Pro Glu Ile Thr Leu Gln Pro Asp Leu Gln
545                 550                 555                 560

Pro Thr Glu Gln Glu Ser Val Ser Leu Trp Cys Thr Ala Asp Lys Ser
                565                 570                 575

Thr Phe Glu Asn Leu Thr Trp Tyr Lys Leu Gly Pro Gln Pro Leu Pro
            580                 585                 590

Val His Val Gly Glu Leu Pro Thr Pro Val Cys Lys Asn Leu Asp Thr
        595                 600                 605

Leu Trp Lys Leu Asn Ala Thr Ile Phe Ser Asn Ser Thr Asn Asp Ile
    610                 615                 620

Leu Ile Met Glu Leu Lys Asn Ala Ser Leu Gln Asp Gln Gly Asp Tyr
625                 630                 635                 640

Val Cys Val Ala Gln Asp Arg Lys Thr Lys Lys Arg His Cys Val Val
                645                 650                 655

Arg Gln Leu Thr Val Leu Glu Arg Val Ala Pro Met Ile Thr Gly Asn
            660                 665                 670

Leu Glu Asn Gln Thr Thr Ser Ile Gly Glu Thr Ile Glu Val Ser Cys
        675                 680                 685

Thr Ala Ser Gly Asn Pro Pro Pro Gln Ile Met Trp Phe Lys Asp Asn
    690                 695                 700

Glu Thr Leu Val Glu Asp Ser Gly Ile Val Leu Lys Asp Gly Asn Arg
705                 710                 715                 720

Asn Leu Thr Ile Arg Arg Val Arg Lys Glu Asp Glu Gly Leu Tyr Thr
                725                 730                 735

Cys Gln Ala Cys Ser Val Leu Gly Cys Ala Lys Val Glu Ala Phe Phe
            740                 745                 750

Ile Ile Glu Gly Ala Gln Glu Lys Thr Asn Leu Glu Ile Ile Ile Leu
        755                 760                 765

Val Gly Thr Ala Val Ile Ala Met Phe Phe Trp Leu Leu Leu Val Ile
    770                 775                 780

Ile Leu Arg Thr Val Lys Arg Ala Asn Gly Gly Glu Leu Lys Thr Gly
785                 790                 795                 800

Tyr Leu Ser Ile Val Met Asp Pro Asp Glu Leu Pro Leu Asp Glu His
                805                 810                 815

Cys Glu Arg Leu Pro Tyr Asp Ala Ser Lys Trp Glu Phe Pro Arg Asp
            820                 825                 830
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```
Arg Leu Lys Leu Gly Lys Pro Leu Gly Arg Gly Ala Phe Gly Gln Val
        835                 840                 845

Ile Glu Ala Asp Ala Phe Gly Ile Asp Lys Thr Ala Thr Cys Arg Thr
    850                 855                 860

Val Ala Val Lys Met Leu Lys Glu Gly Ala Thr His Ser Glu His Arg
865                 870                 875                 880

Ala Leu Met Ser Glu Leu Lys Ile Leu Ile His Ile Gly His His Leu
                885                 890                 895

Asn Val Val Asn Leu Leu Gly Ala Cys Thr Lys Pro Gly Gly Pro Leu
            900                 905                 910

Met Val Ile Val Glu Phe Cys Lys Phe Gly Asn Leu Ser Thr Tyr Leu
        915                 920                 925

Arg Ser Lys Arg Asn Glu Phe Val Pro Tyr Lys Thr Lys Gly Ala Arg
    930                 935                 940

Phe Arg Gln Gly Lys Asp Tyr Val Gly Ala Ile Pro Val Asp Leu Lys
945                 950                 955                 960

Arg Arg Leu Asp Ser Ile Thr Ser Ser Gln Ser Ser Ala Ser Ser Gly
                965                 970                 975

Phe Val Glu Glu Lys Ser Leu Ser Asp Val Glu Glu Glu Glu Ala Pro
            980                 985                 990

Glu Asp Leu Tyr Lys Asp Phe Leu  Thr Leu Glu His Leu  Ile Cys Tyr
        995                 1000                1005

Ser Phe  Gln Val Ala Lys Gly  Met Glu Phe Leu Ala  Ser Arg Lys
    1010                1015                1020

Cys Ile  His Arg Asp Leu Ala  Ala Arg Asn Ile Leu  Leu Ser Glu
    1025                1030                1035

Lys Asn  Val Val Lys Ile Cys  Asp Phe Gly Leu Ala  Arg Asp Ile
    1040                1045                1050

Tyr Lys  Asp Pro Asp Tyr Val  Arg Lys Gly Asp Ala  Arg Leu Pro
    1055                1060                1065

Leu Lys  Trp Met Ala Pro Glu  Thr Ile Phe Asp Arg  Val Tyr Thr
    1070                1075                1080

Ile Gln  Ser Asp Val Trp Ser  Phe Gly Val Leu Leu  Trp Glu Ile
    1085                1090                1095

Phe Ser  Leu Gly Ala Ser Pro  Tyr Pro Gly Val Lys  Ile Asp Glu
    1100                1105                1110

Glu Phe  Cys Arg Arg Leu Lys  Glu Gly Thr Arg Met  Arg Ala Pro
    1115                1120                1125

Asp Tyr  Thr Thr Pro Glu Met  Tyr Gln Thr Met Leu  Asp Cys Trp
    1130                1135                1140

His Gly  Glu Pro Ser Gln Arg  Pro Thr Phe Ser Glu  Leu Val Glu
    1145                1150                1155

His Leu  Gly Asn Leu Leu Gln  Ala Asn Ala Gln Gln  Asp Gly Lys
    1160                1165                1170

Asp Tyr  Ile Val Leu Pro Ile  Ser Glu Thr Leu Ser  Met Glu Glu
    1175                1180                1185

Asp Ser  Gly Leu Ser Leu Pro  Thr Ser Pro Val Ser  Cys Met Glu
    1190                1195                1200

Glu Glu  Glu Val Cys Asp Pro  Lys Phe His Tyr Asp  Asn Thr Ala
    1205                1210                1215
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```
Gly Ile  Ser Gln Tyr Leu Gln  Asn Ser Lys Arg Lys  Ser Arg Pro
    1220                 1225                 1230

Val Ser  Val Lys Thr Phe Glu  Asp Ile Pro Leu Glu  Glu Pro Glu
    1235                 1240                 1245

Val Lys  Val Ile Pro Asp Asp  Asn Gln Thr Asp Ser  Gly Met Val
    1250                 1255                 1260

Leu Ala  Ser Glu Glu Leu Lys  Thr Leu Glu Asp Arg  Thr Lys Leu
    1265                 1270                 1275

Ala Pro  Ser Phe Ser Gly Met  Val Ser Ser Lys Ser  Arg Glu Ser
    1280                 1285                 1290

Val Ala  Ser Glu Gly Ser Asn  Gln Thr Ser Gly Tyr  Gln Ser Gly
    1295                 1300                 1305

Tyr His  Ser Asp Asp Thr Asp  Thr Thr Val Tyr Ser  Ser Glu Glu
    1310                 1315                 1320

Ala Glu  Leu Leu Lys Leu Ile  Glu Ile Gly Val Gln  Thr Gly Ser
    1325                 1330                 1335

Thr Ala  Gln Ile Leu Gln Pro  Asp Ser Gly Thr Thr  Leu Ser Ser
    1340                 1345                 1350

Pro Pro  Val
    1355


<210> SEQ ID NO 102
<211> LENGTH: 98
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 102

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ser Ser Ile Ser Ser Ser Ser Ser Tyr Ile Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg


<210> SEQ ID NO 103
<211> LENGTH: 712
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 103

Met Gln Ser Lys Val Leu Leu Ala Val Ala Leu Trp Leu Cys Val Glu
1               5                   10                  15

Thr Arg Ala Ala Ser Val Gly Leu Pro Ser Val Ser Leu Asp Leu Pro
            20                  25                  30
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-continued

```
Arg Leu Ser Ile Gln Lys Asp Ile Leu Thr Ile Lys Ala Asn Thr Thr
        35                  40                  45

Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu Asp Trp Leu Trp Pro
    50                  55                  60

Asn Asn Gln Ser Gly Ser Glu Gln Arg Val Glu Val Thr Glu Cys Ser
65                  70                  75                  80

Asp Gly Leu Phe Cys Lys Thr Leu Thr Ile Pro Lys Val Ile Gly Asn
                85                  90                  95

Asp Thr Gly Ala Tyr Lys Cys Phe Tyr Arg Glu Thr Asp Leu Ala Ser
            100                 105                 110

Val Ile Tyr Val Tyr Val Gln Asp Tyr Arg Ser Pro Phe Ile Ala Ser
        115                 120                 125

Val Ser Asp Gln His Gly Val Val Tyr Ile Thr Glu Asn Lys Asn Lys
    130                 135                 140

Thr Val Val Ile Pro Cys Leu Gly Ser Ile Ser Asn Leu Asn Val Ser
145                 150                 155                 160

Leu Cys Ala Arg Tyr Pro Glu Lys Arg Phe Val Pro Asp Gly Asn Arg
                165                 170                 175

Ile Ser Trp Asp Ser Lys Lys Gly Phe Thr Ile Pro Ser Tyr Met Ile
            180                 185                 190

Ser Tyr Ala Gly Met Val Phe Cys Glu Ala Lys Ile Asn Asp Glu Ser
        195                 200                 205

Tyr Gln Ser Ile Met Tyr Ile Val Val Val Val Gly Tyr Arg Ile Tyr
    210                 215                 220

Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu
225                 230                 235                 240

Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile
                245                 250                 255

Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu
            260                 265                 270

Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe
        275                 280                 285

Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu
    290                 295                 300

Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr
305                 310                 315                 320

Phe Val Arg Val His Glu Lys Pro Phe Val Ala Phe Gly Ser Gly Met
                325                 330                 335

Glu Ser Leu Val Glu Ala Thr Val Gly Glu Arg Val Arg Ile Pro Ala
            340                 345                 350

Lys Tyr Leu Gly Tyr Pro Pro Pro Glu Ile Lys Trp Tyr Lys Asn Gly
        355                 360                 365

Ile Pro Leu Glu Ser Asn His Thr Ile Lys Ala Gly His Val Leu Thr
    370                 375                 380

Ile Met Glu Val Ser Glu Arg Asp Thr Gly Asn Tyr Thr Val Ile Leu
385                 390                 395                 400

Thr Asn Pro Ile Ser Lys Glu Lys Gln Ser His Val Val Ser Leu Val
                405                 410                 415

Val Tyr Val Pro Pro Gln Ile Gly Glu Lys Ser Leu Ile Ser Pro Val
            420                 425                 430

Asp Ser Tyr Gln Tyr Gly Thr Thr Gln Thr Leu Thr Cys Thr Val Tyr
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        435                 440                 445

Ala Ile Pro Pro Pro His His Ile His Trp Tyr Trp Gln Leu Glu Glu
    450                 455                 460

Glu Cys Ala Asn Glu Pro Ser Gln Ala Val Ser Val Thr Asn Pro Tyr
465                 470                 475                 480

Pro Cys Glu Glu Trp Arg Ser Val Glu Asp Phe Gln Gly Gly Asn Lys
                485                 490                 495

Ile Glu Val Asn Lys Asn Gln Phe Ala Leu Ile Glu Gly Lys Asn Lys
            500                 505                 510

Thr Val Ser Thr Leu Val Ile Gln Ala Ala Asn Val Ser Ala Leu Tyr
        515                 520                 525

Lys Cys Glu Ala Val Asn Lys Val Gly Arg Gly Glu Arg Val Ile Ser
    530                 535                 540

Phe His Val Thr Arg Gly Pro Glu Ile Thr Leu Gln Pro Asp Met Gln
545                 550                 555                 560

Pro Thr Glu Gln Glu Ser Val Ser Leu Trp Cys Thr Ala Asp Arg Ser
                565                 570                 575

Thr Phe Glu Asn Leu Thr Trp Tyr Lys Leu Gly Pro Gln Pro Leu Pro
            580                 585                 590

Ile His Val Gly Glu Leu Pro Thr Pro Val Cys Lys Asn Leu Asp Thr
        595                 600                 605

Leu Trp Lys Leu Asn Ala Thr Met Phe Ser Asn Ser Thr Asn Asp Ile
    610                 615                 620

Leu Ile Met Glu Leu Lys Asn Ala Ser Leu Gln Asp Gln Gly Asp Tyr
625                 630                 635                 640

Val Cys Leu Ala Gln Asp Arg Lys Thr Lys Lys Arg His Cys Val Val
                645                 650                 655

Arg Gln Leu Thr Val Leu Glu Arg Val Ala Pro Thr Ile Thr Gly Asn
            660                 665                 670

Leu Glu Asn Gln Thr Thr Ser Ile Gly Glu Ser Ile Glu Val Ser Cys
        675                 680                 685

Thr Ala Ser Gly Asn Pro Pro Pro Gln Ile Met Trp Phe Lys Asp Asn
    690                 695                 700

Glu Thr Leu Val Glu Asp Ser Glu
705                 710
```

1. An anti-hVEGFR antibody or an antigen-binding fragment thereof, comprising heavy chain HCDR1, HCDR2 and HCDR3 and/or light chain LCDR1, LCDR2 and LCDR3 sequences, wherein:

the HCDR1 sequence comprises SSWMN (SEQ ID NO: 1), DYYMS (SEQ ID NO: 19), X_1YGMS (SEQ ID NO: 41), X_4YWIM (SEQ ID NO: 44);

the HCDR2 sequence comprises RIFPGDGDTYYNGKFQV (SEQ ID NO: 2), FIRNKANGYTTEYSASVKG (SEQ ID NO: 20), SISX$_2$GGSYTYYADSVX$_{19}$G (SEQ ID NO: 42), DIYPGX$_5$GSTNYNEKFKS (SEQ ID NO: 45);

the HCDR3 sequence comprises FLDTSGRYVDY (SEQ ID NO: 3), FDYYGSTYCFDY (SEQ ID NO: 21), EX$_3$DGNYDY (SEQ ID NO: 43), DSNPDY (SEQ ID NO: 46);

the LCDR1 sequence comprises KASQDVNTAVA (SEQ ID NO: 4), RASQSVSTSSSSFMH (SEQ ID NO: 22), RSSKSLLYKDGKTYLN (SEQ ID NO: 28), RASESVX$_6$NSGISFMX$_7$ (SEQ ID NO: 47);

the LCDR2 sequence comprises SASYRYI (SEQ ID NO: 5), YASNLES (SEQ ID NO: 23), LMSTRAS (SEQ ID NO: 29), AASX$_8$QX$_9$S (SEQ ID NO: 48);

the LCDR3 sequence comprises QQHYRAPLT (SEQ ID NO: 6), QHTWEIPLT (SEQ ID NO: 24), QQLVEYPFT (SEQ ID NO: 30), QQSKEVPYT (SEQ ID NO: 49);

wherein X_1 is I or M, X_2 is V or I, X_3 is L or M, X_4 is T or S, X_5 is T or S, X_6 is D or E, X_7 is T or H, X_8 is T or Y, X_9 is G or R, and X_{19} is E or K.

2. The anti-hVEGFR antibody or an antigen-binding fragment thereof of claim **1**, wherein the HCDR1 comprises an amino acid sequence of SEQ ID NO: 41, the HCDR2 comprises an amino acid sequence of SEQ ID NO: 42, the HCDR3 comprises an amino acid sequence of SEQ ID NO: 43, the LCDR1 comprises a sequence of SEQ ID NO: 28, the

LCDR2 comprises a sequence of SEQ ID NO: 29, and the LCDR3 comprises a sequence of SEQ ID NO: 30.

3. The antibody or an antigen-binding fragment thereof of claim **2**, wherein

a) the HCDR1 comprises the sequence of SEQ ID NO: 25, a HCDR2 comprises the sequence of SEQ ID NO: 26, the HCDR3 comprises the sequence of SEQ ID NO: 27; the LCDR1 comprises the sequence of SEQ ID NO: 28, the LCDR2 comprises the sequence of SEQ ID NO: 29, and the LCDR3 comprises the sequence of SEQ ID NO: 30; or

b) the HCDR1 comprises the sequence of SEQ ID NO: 31, the HCDR2 comprises the sequence of SEQ ID NO: 32 or SEQ ID NO: 37, and the HCDR3 comprises the sequence of SEQ ID NO: 33, the LCDR1 comprises the sequence of SEQ ID NO: 28, the LCDR2 comprises the sequence of SEQ ID NO: 29, and the LCDR3 comprises the sequence of SEQ ID NO: 30; or

c) the HCDR1 comprises the sequence of SEQ ID NO: 34, the HCDR2 comprises the sequence of SEQ ID NO: 35 or SEQ ID NO: 37, and the HCDR3 comprises the sequence of SEQ ID NO: 36, the LCDR1 comprises the sequence of SEQ ID NO: 28, the LCDR2 comprises the sequence of SEQ ID NO: 29, and the LCDR3 comprises the sequence of SEQ ID NO: 30.

4. The anti-hVEGFR antibody or an antigen-binding fragment thereof of claim **1**, wherein the HCDR1 comprises an amino acid sequence of SEQ ID NO: 44, the HCDR2 comprises an amino acid sequence of SEQ ID NO: 45, the HCDR3 comprises an amino acid sequence of SEQ ID NO: 46, the LCDR1 comprises a sequence of SEQ ID NO: 47, the LCDR2 comprises a sequence of SEQ ID NO: 48, and the LCDR3 comprises a sequence of SEQ ID NO: 49.

5. The antibody or an antigen-binding fragment thereof of claim **4**, wherein

a) the HCDR1 comprises the sequence of SEQ ID NO: 7, a HCDR2 comprises the sequence of SEQ ID NO: 8, the HCDR3 comprises the sequence of SEQ ID NO:

9; the LCDR1 comprises the sequence of SEQ ID NO: 10, the LCDR2 comprises the sequence of SEQ ID NO: 11, and the LCDR3 comprises the sequence of SEQ ID NO: 12; or

b) the HCDR1 comprises the sequence of SEQ ID NO: 13, the HCDR2 comprises the sequence of SEQ ID NO: 14, and the HCDR3 comprises the sequence of SEQ ID NO: 15, the LCDR1 comprises the sequence of SEQ ID NO: 16, the LCDR2 comprises the sequence of SEQ ID NO: 17, and the LCDR3 comprises the sequence of SEQ ID NO: 18.

6. The antibody or an antigen-binding fragment thereof of claim **1**, wherein

a) the HCDR1 comprises the sequence of SEQ ID NO: 1, the HCDR2 comprises the sequence of SEQ ID NO: 2, and the HCDR3 comprises the sequence of SEQ ID NO: 3, the LCDR1 comprises the sequence of SEQ ID NO: 4, the LCDR2 comprises the sequence of SEQ ID NO: 5, and the LCDR3 comprises the sequence of SEQ ID NO: 6; or

b) the HCDR1 comprises the sequence of SEQ ID NO: 19, the HCDR2 comprises the sequence of SEQ ID NO: 20, and the HCDR3 comprises the sequence of SEQ ID NO: 21, the LCDR1 comprises the sequence of SEQ ID NO: 22, the LCDR2 comprises the sequence of SEQ ID NO: 23, and the LCDR3 comprises the sequence of SEQ ID NO: 24.

7. (canceled)

8. The antibody or antigen-binding fragment thereof of claim **7**, wherein:

the HFR1 comprises a sequence selected from the group consisting of SEQ ID NOs: 64, 68 and 72,

the HFR2 comprises a sequence selected from the group consisting of SEQ ID NOs: 65, 69 and 73,

the HFR3 comprises the sequence selected from the group consisting of SEQ ID NOs: 66, 70 and 74,

the HFR4 comprises a sequence selected from the group consisting of SEQ ID NOs: 67, 71 and 75,

the LFR1 comprises the sequence selected from the group consisting of SEQ ID NOs: 76 and 80,

the LFR2 comprises a sequence selected from the group consisting of SEQ ID NO: 77 and 81,

the LFR3 comprises a sequence selected from the group consisting of SEQ ID NOs: 78 and 82, and

the LFR4 comprises a sequence selected from the group consisting of SEQ ID NOs: 79 and 83.

9. The antibody or an antigen-binding fragment thereof of claim **1**, wherein the heavy chain variable region comprises a sequence selected from the group consisting of SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 98, and a homologous sequence thereof having at least 80% sequence identity yet retaining specific binding affinity to hVEGFR2; and wherein the light chain variable region comprises a sequence selected from the group consisting of SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 96, SEQ ID NO: 97, and a homologous sequence thereof having at least 80% sequence identity yet retaining specific binding affinity to hVEGFR2.

10. (canceled)

11. The antibody or an antigen-binding fragment thereof of claim **1**, wherein:

a) a heavy chain variable region comprising the sequence of SEQ ID NO: 50 and a light chain variable region comprising the sequence of SEQ ID NO: 51;

b) the heavy chain variable region comprises a sequence of SEQ ID NO: 52 and the light chain variable region comprises a sequence of SEQ ID NO: 53;

c) the heavy chain variable region comprises a sequence of SEQ ID NO: 54 and the light chain variable region comprises a sequence of SEQ ID NO: 55;

d) the heavy chain variable region comprises a sequence of SEQ ID NO: 56 and the light chain variable region comprises a sequence of SEQ ID NO: 57;

e) the heavy chain variable region comprises a sequence of SEQ ID NO: 58 and the light chain variable region comprises a sequence of SEQ ID NO: 59;

f) the heavy chain variable region comprises a sequence of SEQ ID NO: 60 and the light chain variable region comprises a sequence of SEQ ID NO: 61;

g) the heavy chain variable region comprises a sequence of SEQ ID NO: 62 and the light chain variable region comprises a sequence of SEQ ID NO: 63; or

h) the heavy chain variable region comprises a sequence of SEQ ID NO: 93 or SEQ ID NO: 94 or SEQ ID NO:

98 and the light chain variable region comprises a sequence of SEQ ID NO: 96 or SEQ ID NO: 97.

12-13. (canceled)

14. The antibody or antigen-binding fragment thereof of claim **1**, further comprising an immunoglobulin constant region, or further comprising a constant region of human Ig, or further comprising a constant region of human IgG.

15. (canceled)

16. The antibody or antigen-binding fragment thereof of claim **15**, wherein the constant region of human IgG is human IgG1 comprising SEQ ID NO: 38, or a homologous sequence having at least 80% sequence identity thereof.

17. The antibody or an antigen-binding fragment thereof of claim **1**, which is humanized.

18. The antibody or antigen-binding fragment thereof of claim **1**, which is a diabody, a Fab, a Fab', a $F(ab')_2$, a Fd, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), an scFv dimer (bivalent diabody), a multispecific antibody, a camelized single domain antibody, a nanobody, a domain antibody, or a bivalent domain antibody.

19. The antibody or antigen-binding fragment thereof of claim **1**, which is bispecific, capable of specifically binding to a first and a second epitope of hVEGFR2, or capable of specifically binding to hVEGFR2 and a second antigen.

20. (canceled)

21. The antibody or antigen-binding fragment thereof of claim **19**, wherein the second antigen is an immune related target or a tumor antigen, wherein the immune related target is selected from the group consisting of PD-L1, PD-L2, PD-1, CLTA-4, TIM-3, LAG3, CD160, 2B4, TGF β, VISTA, BTLA, TIGIT, LAIR1, OX40, CD2, CD27, ICAM-1, NKG2C, SLAMF7, NKp80, CD160, B7-H3, LFA-1, 1COS, 4-1BB, GITR, CD30, CD40, BAFFR, HVEM, CD7, LIGHT, IL-2, IL-15, CD3, CD16 and CD83, and wherein the tumor antigen is selected from the group consisting of, claudin 18.2, CA-**125**, gangliosides G(D2), G(M2) and G(D3), CD20, CD52, CD33, Ep-CAM, CEA bombesin-like peptides, PSA, HER2/neu, epidermal growth factor receptor (EGFR), erbB2, erB3/HER3, erbB4, CD44v6, Ki-67, cancer-associated mucin, VEGF, VEGFRs (e.g., VEGFR3), estrogen receptors, Lewis-Y antigen, TGFβ1, IGF-1 receptor, EGFα, c-Kit receptor, transferrin receptor, IL-2R and CO17-1A.

22-24. (canceled)

25. The antibody or antigen-binding fragment thereof of claim **1** linked to one or more conjugate moieties, wherein the conjugate moiety comprises a clearance-modifying agent, a chemotherapeutic agent, a toxin, a radioactive isotope, a lanthanide, a luminescent label, a fluorescent label, an enzyme-substrate label, a DNA-alkylators, a topoisomerase inhibitor, a tubulin-binders, or other anticancer drugs.

26-27. (canceled)

28. A pharmaceutical composition comprising the antibody or antigen-binding fragment thereof of claim **1**, and one or more pharmaceutically acceptable carriers.

29-30. (canceled)

31. An isolated polynucleotide encoding the antibody or an antigen-binding fragment thereof of claim **1**.

32. A vector comprising the isolated polynucleotide of claim **31**.

33. A host cell comprising the vector of claim **32**.

34. A method of expressing the antibody or antigen-binding fragment thereof of claim **1**, comprising culturing a host cell comprising a vector comprising an isolated polynucleotide encoding the antibody o antigen-binding fragment thereof of claim **1** under the condition at which the vector is expressed.

35. A method of treating, reducing the severity of and/or slowing the progression of a VEGFR2-related disease or condition in a subject, comprising administering to the subject a therapeutically effective amount of the antibody or antigen-binding fragment thereof of claim **1**.

36. The method of claim **35**, wherein the VEGFR2-related disease or condition is a tumor or an angiogenic disease.

37. The method of claim **36**, wherein the tumor produces VEGF (e.g., VEGF-A) and/or is sensitive to VEGF (e.g., VEGF-A) present in its microenvironment.

38. (canceled)

39. The method of claim **36**, wherein the tumor is selected from the group consisting of breast carcinoma, lung carcinoma, colorectal carcinoma, pancreatic carcinoma, glioma and lymphoma, head and neck tumors, neuroendocrine tumors, colorectal tumors, prostate tumors, breast tumors, lung tumors, such as small cell and non-small cell lung tumors, pancreatic tumors, thyroid tumors, ovarian tumors, liver tumors, Kaposi's sarcoma, CNS neoplasms, neuroblastomas, capillary hemangioblastomas, meningiomas, cerebral metastases, melanoma, gastrointestinal and renal carcinomas and sarcomas (e.g., gastric cancer), rhabdomyosarcoma, glioblastoma, preferably glioblastoma multiforme, leiomyosarcoma, squamous cell carcinoma, basal cell carcinoma and skin cancers that can be treated by suppressing the growth of malignant keratinocytes, such as human malignant keratinocytes, and wherein the angiogenetic disease is selected from the group consisting of atherosclerosis, rheumatoid arthritis (RA), neovascular glaucoma, proliferative retinopathy including proliferative diabetic retinopathy, macular degeneration, hemangiomas, angiofibromas, psoriasis, retinopathy of prematurity (e.g., retrolental fibroplastic), corneal graft rejection, insulin-dependent diabetes mellitus, multiple sclerosis, myasthenia gravis, Chron's disease, autoimmune nephritis, primary biliary cirrhosis, acute pancreatitis, allograph rejection, allergic inflammation, contact dermatitis, and delayed hypersensitivity reactions, inflammatory bowel disease, septic shock, osteoporosis, osteoarthritis, cognition defects induced by neuronal inflammation, Osler-Weber syndrome, restinosis, and fungal, parasitic and viral infections, such as cytomegaloviral infections.

40-42. (canceled)

43. The method of claim **35**, wherein the subject is human.

44-47. (canceled)

48. A method of detecting presence or amount of VEGFR2 in a sample, comprising contacting the sample with the antibody or antigen-binding fragment thereof of claim **1**, and determining the presence or the amount of VEGFR2 in the sample.

49-53. (canceled)

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